IMMUNE MODULATORY EFFECT OF HYPERTHERMIA DURING TREATMENT OF RECURRENT, METASTATIC OR LOCALLY ADVANCED SOLID ORGAN MALIGNANCIES

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Abstract

Background: The use of hyperthermia as an adjunct to improve conventional therapies in multimodal cancer treatment is supported by an increasing body of research. The underlying biological contribution of hyperthermia in modulating the cells of the immune system has been a growing area of interest. This study aims to evaluate the immune modulatory effect of locoregional hyperthermia given with standard of care treatment for patients with recurrent or metastatic solid malignancies. Secondary endpoints were tolerability, change in pain score and quality of life, and tumour control rate.

Methods: A single-centre prospective study, conducted from December 2019 to December 2021 at the University of Malaya Medical Centre, recruited 30 patients with solid organ malignancy at baseline. Alongside standard cancer treatment, patients also received hyperthermia treatment for 2 hours, two or three times a week for up to 16-weeks using REMISSION1ºC hyperthermia-induction device. Flow cytometry for lymphocyte enumeration and immunophenotyping was done on blood samples collected from patients at 4 different time points (Baseline, week 1 post hyperthermia, week 1 post standard therapy, week 4.

Results: In the analysis of lymphocyte subsets, an increase in CD8+ central memory T cells between baseline and week 1 post hyperthermia (Mean: Baseline 3.854, Week 1A 5.818; P = 0.013) was noted. An increase of CD4+ effector memory T cells between baseline and week 4 post hyperthermia in conjunction with standard therapy (Mean: Baseline 33.60, Week 4 39.34; P = 0.022) was recorded. There was also a marked increase in the percentage of CD8+ T cell expressing checkpoint inhibitory marker PD-1 post week 1 hyperthermia treatment (Mean: Baseline 7.439, week 1A 9.757; P = 0.048) as well as post standard treatment (Mean: Baseline 7.439, Week 1B 9.222; P = 0.033) from baseline.

Conclusion: Hyperthermia produced significant effects on the immune cell profiles of cancer patients on treatment. The subset of CD4 effector memory T cells increased significantly, although there was also sign of increased T cells exhaustion. These findings serve as a stepping stone for further research in exploring the mechanisms of hyperthermia in immune modulation and also its translation into clinical practice.

Keywords: Hyperthermia, Advanced Malignancy, Immune Modulation

Introduction

Hyperthermia is a non-invasive treatment which involves selective heating of tumour tissue to temperatures ranging between 39°C to 43°C. It is generally applied as an adjunct to established non-surgical cancer treatments, such as chemotherapy and radiotherapy. Studies have demonstrated that hyperthermia is a potent radiosensitizer (1), chemosensitizer (2) and immunomodulator (3–5) and when used in conjunction with standard oncological treatment improves tumour response and overall survival for multiple tumour sites (6–9). In addition, current technological advancements in hyperthermia delivery system have led to more potent and safer treatments.

Multiple in vitro and in vivo studies have explored the mechanisms contributing to the therapeutic effects of hyperthermia in the treatment of cancer, which includes

inhibition of DNA damage repair, increased blood flow and vascular permeability and tumour oxygenation (10, 11). Furthermore, recent literature also indicate a distinctive immunomodulating prospect of hyperthermia (3, 4, 11–15). There is growing interest in understanding the role of hyperthermia in the modulation of various arms of the immune system in cancer patients. Understanding these intrinsic mechanisms could bring new light onto the analysis of current clinical trials as well as designing future trials in the use of hyperthermia for cancer treatment.

In this study, we aim to evaluate the immune modulatory effect of locoregional hyperthermia given with standard of care treatment for patients with recurrent or metastatic solid malignancies. Secondary endpoints were tolerability of hyperthermia, change in pain score and quality of life, and tumour control rate.

Materials and Methods

Study oversight

This single-centre prospective study was conducted from Dec 2019 to Dec 2021 at the University of Malaya Medical Centre, a tertiary referral hospital with oncological services in Malaysia. Data cut-off was on 1st of Dec 2021. Study approval was granted by the Medical Research Ethics Committee at the University of Malaya Medical Centre. This study was conducted in accordance with the World Medical Association Declaration of Helsinki and Malaysian Guidelines for Good Clinical Practice and applicable regulatory requirements. All participants provided written, informed consent prior to any study procedures.

Study objectives

The primary outcome of this study was to evaluate the immune response of locoregional hyperthermia given with standard of care cancer treatment in recurrent, metastatic or locally advanced solid malignancies. The secondary outcomes were to assess the tolerability of hyperthermia, change in pain score and quality of life, and tumour control rate.

Patients

Eligible patients had a histologically confirmed solid malignancy at baseline; were planned for cancer treatment, age ≥ 18, performance status of 0-2, had life expectancy longer than 4 weeks, tumor measurable by response evaluation criteria in solid tumors (RECIST) criteria, cardiovascular: resting ventricular ejection fraction greater than 40%, neurology: stable treated brain metastases, able to communicate verbally. Patients were excluded from study entry if they were pregnant, had coagulopathy, symptomatic coronary artery disease, active thromboembolic disease, cardiac arrhythmia, uncontrolled seizures, mentally confused or had electronic devices such as artificial heart, pacemaker etc. A written informed consent prior to participation was a requirement.

Study design

Patients with recurrent, metastatic, or locally advanced solid malignancy at baseline who were planned for cancer treatment were recruited and received both hyperthermia therapy alongside standard of care cancer management as per planned by the primary treating physician.

Hyperthermia therapy was delivered via the HYPERTHERMIA-REMISSION1ºC device, which uses radiofrequency field that leads to vibration of ions in the target tissue via dielectric heating, and electric resistance increases the internal temperature via joule heating. It operates at relatively lower frequency, 0.46 MHz, whereas the existing studies used at least 1 MHz, leading to deeper penetration and less surface heating, compared to conventional RF hyperthermia machine.

During standard cancer treatment, patients were scheduled to receive hyperthermia treatment for 2 hours, two or three times a week using REMISSION1ºC hyperthermia-induction device while awake, under no sedation, in a comfortable position at rest. The hyperthermia delivery process was monitored by an attendant during the entire process. The treatment and assessment were continued up to 16 weeks.

During the treatment period, patients were reviewed weekly prior to their hyperthermia treatment by the treating physician. Blood investigations (Full blood count with differentials, renal function, liver function test, lactate dehydrogenase, C-reactive protein, erythrocyte sedimentation rate, coagulation profile) were performed during screening and at 3 weekly intervals. At each treatment, patients were rated for toxicity using the National Cancer Institute's Common Terminology Criteria for Adverse Events version 4.03 (NCI-CTCAE v4.03).

Immune modulatory effects of hyperthermia were assessed using immunology cells analysis, including both lymphocyte (NK, B and T cell) enumeration and immunophenotyping. The analysis was performed at 4 time points; during screening (Baseline), after 1st hyperthermia treatment prior to standard treatment (Week 1A), after 1st standard treatment (Week 1B), and after 4 weeks of hyperthermia treatment alongside standard therapy (Week 4).

For NK, B and T cell enumeration, 3mL of whole blood were collected in 1 EDTA Tube and sent to Immunology Lab in University Malaya Medical Centre. NK, T and B cell enumeration were performed by flow cytometry and included the measurements for CD4 T cells, CD8 T cells and Natural Killer cells.

For lymphocyte immunophenotyping and other immune analysis, 30mL of whole blood were collected in 3 EDTA tubes and sent to the Immunotherapeutics Laboratory, University Malaya Medical Centre. Lymphocyte immunophenotyping was performed on whole blood by flow cytometry and included measurements of activation, checkpoint and functional markers of lymphocyte subsets (CD4 and CD8 T cells, NK cells).

Pain score was evaluated using the Edmonton Symptom Assessment System: Numerical Scale. Quality of life was evaluated with the EORTC QLQ C30 questionnaire. These assessments were performed at baseline, then weekly for 8 weeks.

Response to treatment was assessed by the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST). Baseline CT scan was done within 1 month of study commencement (date of consent), then every 9 weeks for reassessment, until standard treatment was completed or upon investigator judgement.

Patients were followed every 4 weeks until death or until 6 months from first hyperthermia treatment, whichever occurs first. (Refer to Appendix 1 for evaluation and visit schedule)

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test before exploring Wilcoxon sum rank test. Statistical analysis of all the immune markers between baseline to Week 1A, Week 1B, and Week 4 were performed using the related samples Wilcoxon-signed rank test using built-in functions in Program R version 3.6.0 displaying the mean and p-value, as illustrated in Table 1. A probability value $p < 0.05$ was considered to be statistically significant in all the analysis done.

Results

Patient characteristics

Thirty patients consented to be enrolled into the study and underwent hyperthermia treatment as per protocol.

Baseline characteristics of the thirty patients are summarized in Table 1. Median age of patients was 54 years, ranging from 23 to 72 years old. Majority of the patients were female (70%) and Chinese (93.3%). The most common tumor site was breast (n = 9, 29%) followed by colorectal (n = 8, 25.8%). One of the patients enrolled had dual primary disease with both ovarian and pancreatic cancer. 70% of patients had metastatic disease upon participation (n = 21), 20% had recurrent disease (n = 6), 10% had locally advanced disease (n = 3). 63.3% of patients had Eastern Cooperative Oncology Group (ECOG) performance status of 0 (n = 19), 26.7% and 10% had ECOG status of 1 and 2 respectively. 33.3% of patients had radiotherapy (n = 10), 80% of patients had chemotherapy (n = 24). 30% of patients were receiving the second and fourth line of cancer treatment during the period of this study (n=9), followed by 20% on their first (n = 6), and 10% on fifth line of cancer treatment (n = 3).

Table 1: Baseline characteristics of patients (n = 30)

Characteristics	Estimates*
Age, *years*	54 (23 – 72)
Sex	
Male	9 (30)
Female	21 (70)
Ethnicity	
Chinese	28 (93.3)
Malay	1 (3.3)
Others	1 (3.3)
Tumor Sites	
Breast	9 (29)
Colorectal	8 (25.8)
Pancreas	3 (9.7)
Lung	2 (6.5)
Ovary	3 (9.7)
Head and neck	3 (9.7)
Sarcoma	2 (6.4)
Neuroendocrine	1 (3.2)
Staging	
Locally advanced	3 (10)
Metastatic	21 (70)
Recurrent	6 (20)
ECOG	
0	19 (63.3)
1	8 (26.7)
2	3 (10)
Radiotherapy	
Yes	10 (33.3)
No	20 (66.7)
Systemic therapy	
Yes	24 (80)
No	6 (20)
Line of treatment	
1st	6 (20)
2nd	9 (30)
3rd	2 (6.7)
4th	9 (30)
5th	3 (10)
> 5	1 (3.3)

*Estimates are reported as Median (range) for age and N (%) for categorical variables

Immune response

Statistical analysis using Wilcoxon-signed rank test displaying the mean and p-value comparing all the immune markers between baseline to week1A, week1B and Week4 respectively were summarized in Table 2. A probability value of $p < 0.05$ shows statistical significance.

From baseline to week 1A (post hyperthermia treatment), a significant increase in the percentages of CD8+ central

memory T cells (Mean: Baseline 3.854, Week 1A 5.818; P = 0.013) and CD8+ T cell expressing checkpoint inhibitory marker PD-1 (Mean: Baseline 7.439, week 1A 9.757; P = 0.048).

There was also a marked increase in the percentage of CD8+ T cell expressing checkpoint inhibitory marker PD-1 (Mean: Baseline 7.439, Week 1B 9.222; P = 0.033) noted at time point Week 1B (Week 1 post standard treatment) from baseline.

At week 4 of treatment (4 weeks of treatment with hyperthermia and standard therapy) an increase in the percentage of CD4+ effector memory T cells (Mean: Baseline 33.60, Week 4 39.34; P = 0.022) were observed.

Table 2: Statical analysis of all the immune markers between baseline to week1A, baseline to Week1B, baseline to Week 4 was performed using Wilcoxon-signed rank test. Wilcoxon-signed rank sum test was used to compare the immune response between baseline to week1A, baseline to Week1B, baseline to Week 4 displaying the mean and p-value (Table 1). A probability value $p < 0.05$ shows statistical significance.

Markers	Mean				p-value		
	Baseline	Week 1A	Week 1B	Week 4	Week 1A	Week 1B	Week 4
Cytotoxic NK cells (CD56 dim CD16 bright)	18.18	22.957	19.171	24.650	0.1482	0.8195	0.07629
Cytotoxic NK cells expressing NKp46+	32.825	32.711	34.09	28.500	0.3684	0.811	0.7282
Cytotoxic NK cells expressing CD94+	45.62	46.51	49.16	57.05	0.857	0.8195	0.5546
Cytotoxic NK cells expressing NKG2D	54.89	56.53	54.88	56.47	0.6736	0.9184	0.6021
Cytotoxic NK cells expressing P2 Perforin	67.25	59.10	68.74	62.04	0.2977	0.8823	0.3506
Cytotoxic NK cells expressing Granzyme B	71.73	65.18	68.61	66.13	0.1987	0.1671	0.0702
Regulatory NK cells (CD56 bright CD16 dim/-)	1.204	0.9464	0.8821	0.9591	0.2318	0.317	0.2303
Regulatory NK cells expressing NKp46+	71.03	72.46	67.14	76.31	0.5769	0.3109	0.357
Regulatory NK cells expressing CD94+	69.32	74.21	68.55	77.00	0.2022	0.6902	0.2238
Regulatory NK cells expressing NKG2D+	69.50	71.54	65.74	73.35	0.374	0.4564	0.6143
Regulatory NK cells expressing Perforin	21.14	27.18	19.69	31.636	0.1747	0.9003	0.2586
Regulatory NK cells expressing Granzyme B	42.19	40.92	38.93	50.81	0.8949	0.4004	0.1099
CD4+ T Cells	51.67	53.35	52.15	50.26	0.9909	0.9521	0.871
CD4+ Central Memory T Cells	19.57	19.85	19.37	15.86	0.7071	0.414	0.1192
CD4+ Naïve T Cells	32.51	34.70	33.44	29.85	0.4732	0.9595	0.1153
CD4+ Effector Memory T Cells	33.60	34.19	35.19	39.34	0.6736	0.5165	0.02207**
CD4+ Effector Memory T Cells re-expressess CD45RA	14.339	11.243	12.00	14.941	0.3806	0.3878	0.2989
CD4+ T cells expressing Checkpoint Inhibitory Marker PD-1	7.386	8.582	9.111	7.318	0.2743	0.07738	0.871
CD4+ T cells expressing Activation marker HLADR	18.386	18.018	16.75	20.686	0.7156	0.7007	0.6263
CD4+ T cells expressing Homing Marker CD62L	47.55	55.05	53.07	42.51	0.09415	0.4637	0.3986
CD8+ T Cells	44.65	41.29	43.17	45.18	0.2949	0.6294	0.8456
CD8+ Central Memory T Cells	3.854	5.818	5.822	5.855	0.01348**	0.06554	0.1443
CD8+ Naïve T Cells	27.61	31.53	31.03	29.58	0.4456	02531	1.000
CD8+ Effector Memory T Cells	37.11	35.93	36.55	34.97	0.8913	0.9139	0.6034
CD8+ Effector Memory T Cells re-expressess CD45RA	29.82	28.45	26.60	29.59	0.3219	0.1213	0.871
CD8+ T cells expressing Checkpoint Inhibitory Marker PD-1	7.439	9.757	9.222	7.645	0.04886**	0.03348**	0.9446
CD8+ T cells expressing Activation marker HLADR	44.97	45.48	42.53	42.62	0.7071	0.4072	0.4954
CD8+ T cells expressing Homing Marker CD62L	28.29	31.34	33.88	28.90	0.6987	0.2538	0.721
Regulatory T Cells	3.93	4.454	4.186	5.087	0.218	0.648	0.1116
Regulatory T Cells expressing CD39+	48.58	44.18	44.16	44.42	0.1987	0.08152	0.6968
Regulatory T Cells expressing CTLA-4+	0.6667	0.5714	0.7429	0.3957	0.6265	0.4452	0.2189
Regulatory T Cells expressing CD103+	2.13	2.221	2.068	2.122	0.7185	0.9678	0.6967

Tolerability and adherence

Overall pain score assessed using the Edmonton Symptom Assessment System: Numerical Scale showed improvement over the weeks of treatment, as illustrated in Figure 1. Highest pain score at baseline was 4. After week 3 of treatment, there were no more patients with a recorded pain score of 4 and above.

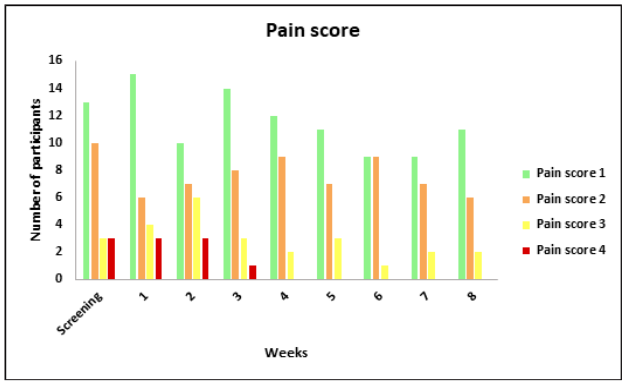

Figure 1: Pain score based on Edmonton Symptom Assessment System: Numerical Scale over 8 weeks. A higher pain score indicating greater pain.

Quality of life score assessed using EORTC QLQ-C30 questionnaire generally demonstrated improvement with an overall increasing mean score throughout the 8 weeks of assessment, as shown in Figure 2.

Figure 2: Quality of life score based on EORTC QLQ-C30 over 8 weeks. Error bars represent standard errors of the mean. A higher score represents better quality of life.

The median number of total hyperthermia treatments per patient is 20.5, with the minimum of 2 and a maximum of 37.

A total of 14 drop-outs were reported over the course of 16 weeks. Reasons stated for discontinuation of hyperthermia treatment were consent withdrawal (n = 4), adverse reaction (n = 4), progressive disease (n = 3), death (n = 3). Most common reported reason for withdrawal of consent was that patients were not keen for the frequent hospital visits required for hyperthermia treatment.

Hyperthermia-related adverse events

Grade 1-2 hyperthermia-related toxicities were observed in 16.7% of patients during hyperthermia treatment (n = 5). The most common toxicity seen was skin burn (n = 3) followed by subcutaneous fat necrosis (n = 2). Most common manifestation was pain. One patient with G1 fat necrosis and another with G2 skin burn subsequently discontinued treatment. All adverse events healed spontaneously without any clinical intervention.

Adverse events

All 30 patients were evaluable for toxicity. Grade 3 adverse events were reported in 2 out of the 30 patients. One patient developed lung infection requiring admission and intravenous antibiotic therapy. Another patient suffered from minor trauma following an alleged fall at home.

There were three reported deaths during treatment period and three reported deaths during the subsequent 6 month follow up period.

Of the reported deaths during the treatment period, one of the patients had underlying recurrent right breast cancer with extensive bone, liver, lung metastases failing 4 lines of treatment and cause of death reported was disease progression. Another patient had metastatic lung cancer in disease progression with lymphangitis carcinomatosis and cause of death was reported to be severe pneumonia. Third reported death was a patient with underlying metastatic osteosarcoma with extensive lung metastases on the 3rd line of palliative treatment that passed away following a bout of severe pneumonia with pneumothorax and pulmonary embolism.

The three other reported deaths during the subsequent 6 month follow up period were due to disease progression of underlying breast, lung and colon cancer respectively.

Radiological response

Based on the overall best RECIST criteria response and intention-to-treat analysis, the disease control rate across 30 enrolled patients were 43.3% with 13 patients showing stable disease and 2 patients with partial response. 20% of patients had progressive disease (n = 6) and 36.7% were not evaluable (n = 11). Patients who were not evaluable were due to non-compliance, consent withdrawal or death.

Discussion

In our study, we focused on assessing the effect of hyperthermia on NK cells, B cells and T cells. We observed an increase in percentages of CD8+ central memory T cells as well as the CD4+ effector memory T cells. CD8 central memory T cells play a pivotal role in cancer immunity, characterized by their capacity for long-term persistence, rapid recall responses, and crucial contributions to tumor

surveillance. CD4 effector memory T cells in cancer demonstrate heightened effector functions, including cytokine secretion and rapid response capabilities, playing pivotal roles in orchestrating anti-tumor immune responses within the tumor microenvironment. Several studies have demonstrated that both central and effector memory CD8+ T cell subsets mediate long-lived anti-tumor immunity (16, 17).

However, a significant increase was also seen in the percentages of CD8+ T cell expressing checkpoint inhibitory marker PD-1 post hyperthermia therapy. This increment was noted to be sustained following the administration of standard treatment as well. CD8+ T cell expressing checkpoint inhibitory marker PD-1 are well established in the inhibitory pathway of T-cell anti-tumour activity in physiological contexts (18). CD8 T cells expressing the checkpoint inhibitory marker PD-1 in the context of cancer often exhibit a state of functional exhaustion, hindering their cytotoxic activity and impeding effective anti-tumor immune responses. However, there are some new data to suggest that PD-1 expression is first a marker of T cell activation. Thus, its role remains ambiguous in defining effective or ineffective immune T cell responses (19, 20).

There were no significant changes observed in both NK and B cells throughout the study. However, it may be important to take note that it was serum NK cell levels that were measured in this study, whereas it is possible that NK cells may increase more significantly in the tumor microenvironment which may more accurately reflect the expected immune response.

The benefit of pain control with hyperthermia treatment has been shown in multiple studies (21, 22). Although there were no statistically significant changes seen in both pain score and quality of life scores in our study population, there was a notable reduction in the groups with pain score > 4 over the course of treatment.

There were no serious adverse events related to hyperthermia treatment throughout this study. Grade 1-2 skin burn, fat necrosis was noted with pain being the most common presentation. However, it was noted that the drop-out rates were significant for this study with the most common reported reason being consent withdrawal and adverse reaction. Reasons quoted for consent withdrawal were mostly issues surrounding the time commitment, frequent hospital visits for hyperthermia treatment and long treatment period. Therefore, it is crucial for treating physicians to take into account patient's goal of therapy and commitments to be able to come up with a comprehensive patient-specific hyperthermia treatment plan.

Several limitations should be kept in mind in considering the clinical significance of our findings. This was a single institutional cohort with a small sample size and a group of heterogenous study population with varying primary tumor sites undergoing widely different standard treatment modalities. Thus, it needs to be considered that the immune and disease response shown above may not be solely attributable to hyperthermia treatment. A randomized comparative study of a less heterogenous group of patients may be beneficial to help ascertain if the immune response was attributable to primary disease, standard treatment or hyperthermia itself.

Conclusion

Hyperthermia produced significant effects on the immune cell profiles of cancer patients on treatment. The subset of CD4 effector memory T cells increased significantly, although there was also sign of increased T cells exhaustion. These findings serve as a stepping stone for further research in exploring the mechanisms of hyperthermia in immune modulation and also its translation into clinical practice.

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Competing interests

The authors declare no competing financial interests or conflicts of interest related to this work.

Ethical Clearance

We obtained approval from the Medical Research and Ethics Committee (MREC) and the Ministry of Health Malaysia (MOH), registered under NMRR-19-2585-47819.

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Appendix

Appendix 1: Evaluation and visit schedule

	Screening	Week 1, 2, 3 and 4[a]			EOT	Follow up
		Treatment 1	Treatment 2	Treatment 3		
Informed consent	X					
Medical history	X					
Inclusion and exclusion criteria	X					
Full Physical Examination[d]	X	X				
Vital signs and weight[e]	X	X				
Concomitant medications notation[f]	X	X				
AEs monitoring[g,]			X			X[f,h]
ECOG performance status	X	X				
Haematology (Full Blood Count with differential)[i]	X	X			X	
Chemistry[j]	X	X			X	
ESR[j]	X	X			X	
CRP[j]	X	X			X	
PT/INR[k]	X	X			X	
Serum pregnancy test[l]	X					
Immunology Cells Analysis (Lymphocytes Enumeration and Immunophenotyping)	X	X[m]				
12-Lead Electrocardiogram	X				X	
Imaging[n]	X	X				
REMISSION 1℃ hyperthermia-induction therapy[o]		X	X	X		
Patient Reported Outcomes[p]	X	X				
Survival status						X

a. Treatment will be continued up to 16 weeks from Day 1 of Hyperthermia treatment.

b. An End of Treatment (EOT) visit will occur 3 weeks after the last dose of study treatment regardless of the reason of discontinuation.

c. All patients will be followed every 4 weeks until death or until 6 months from Treatment 1 of Week 1, whichever occurs first.

d. Full physical examination and directed physical examination will include neurologic examination to be performed by the treating physician or designee.

e. Vital signs (including blood pressure, heart rate and temperature) will be assessed prior to study treatment administration and be measured after 5 minutes of rest (sitting). Height will be assessed at Screening only as a baseline measurement.

f. Record all medication taken within 30 days prior to initiation of treatment and all medication taken during treatment period of the study.

g. Adverse events will be assessed from the date of informed consent form is signed until up to 28 days from the last dose of study treatment, regardless of the relationship to the study drug. Where an AE is ongoing at the EOT visit, the AE will be followed until one of the following: resolution or improvement from baseline, relationship reassessed as unrelated, start of new anti-cancer therapy, confirmation from the investigator that no further improvement can be expected, end of collection of clinical or safety data, or final database closure. For patients who do not enter the Survival Follow-Up Period, the last dose assessed status of AEs will be collected.

h. SAEs related to study treatment occurring during the Survival Follow-Up Period will be reported and followed up.

i. Haematology (Full Blood Count with differential) and Chemistry (LFT, RFT, AST, LDH) for Week 1 will be performed within 14 days prior to Treatment 1 of Week 1. For all other cycles, Haematology (Full Blood Count with differential) and Chemistry (LFT, RFT, AST,

LDH) will be performed in 3 weekly interval within 3 days prior to Treatment 1 of each cycle.

j. CRP and ESR test will be performed in 3 weekly interval. CRP and ESR test for Week 1 will be performed within 14 days prior to Treatment 1 of Week 1.

k. PT/INR for Week 1 will be performed within 14 days prior to Treatment 1 of Week 1 and within 3 days prior to Treatment 1 of Week 2 and when clinically indicated for the patients who has been administered aspirin.

l. Serum pregnancy test should be performed at the Screening (within 3 days prior to Treatment 1 of Week 1), or at any time if pregnancy is suspected in females of childbearing potential only. For the screening visit, only patients who have confirmed negative pregnancy test results will be included in this study.

m. Blood for Lymphocytes Enumeration and Immunophenotyping analysis will be taken after Standard Treatment (Week 1) and after Hyperthermia Treatment 1 (Week 1 and Week 4) (within an hour, or otherwise approved by Immunology laboratory).

Lymphocyte Enumeration:

i. Screening

ii. Post-Hyperthermia (Week 1)

iii. Post-Standard Treatment (Week 1)

iv. Post Hyperthermia (Week 4)

Immunophenotyping:

i. Screening

ii. Post-Hyperthermia (Week 1)

iii. Post-Standard Treatment (Week 1)

iv. Post Hyperthermia (Week 4)

n. Imaging assessment will be done within one month of study commencement, then every 9 weeks from baseline.

o. REMISSION 1℃ hyperthermia-induction therapy will be applied for 2 hours, two or three times a week.

p. Patient Reported Outcomes include EORTCQLQ-C30 and Symptom Assessment Scale need to be answered by subjects at baseline, then weekly for 8 consecutive weeks.