

Perspective

## Oncology Acupuncture Clinical Trials: proposal of a new approach

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### Abstract:

Recently the quantity of clinical trials on oncology acupuncture has been increased due to the gaining of popularity amongst cancer patients, mostly to relieve side effects of conventional cancer treatment. Several limitations to acupuncture trials have been pointed out which may be due to the fact that most clinical research are focused on symptoms management. This paper proposes a new approach on acupuncture clinical trials for cancer patients undergoing chemotherapy that might allow the assessment of acupuncture effects on homeostasis as well as to interpret mechanisms by which single or multiple symptoms may be relieved and to establish a standardized acupuncture protocol for cancer patients undergoing chemotherapy.

**Keywords:** Acupuncture; Chemotherapy; Oncology; Immunity; Research; Methodology; Clinical Trials; Neuroimmunovegetative approach; Psychoneuroimmunology

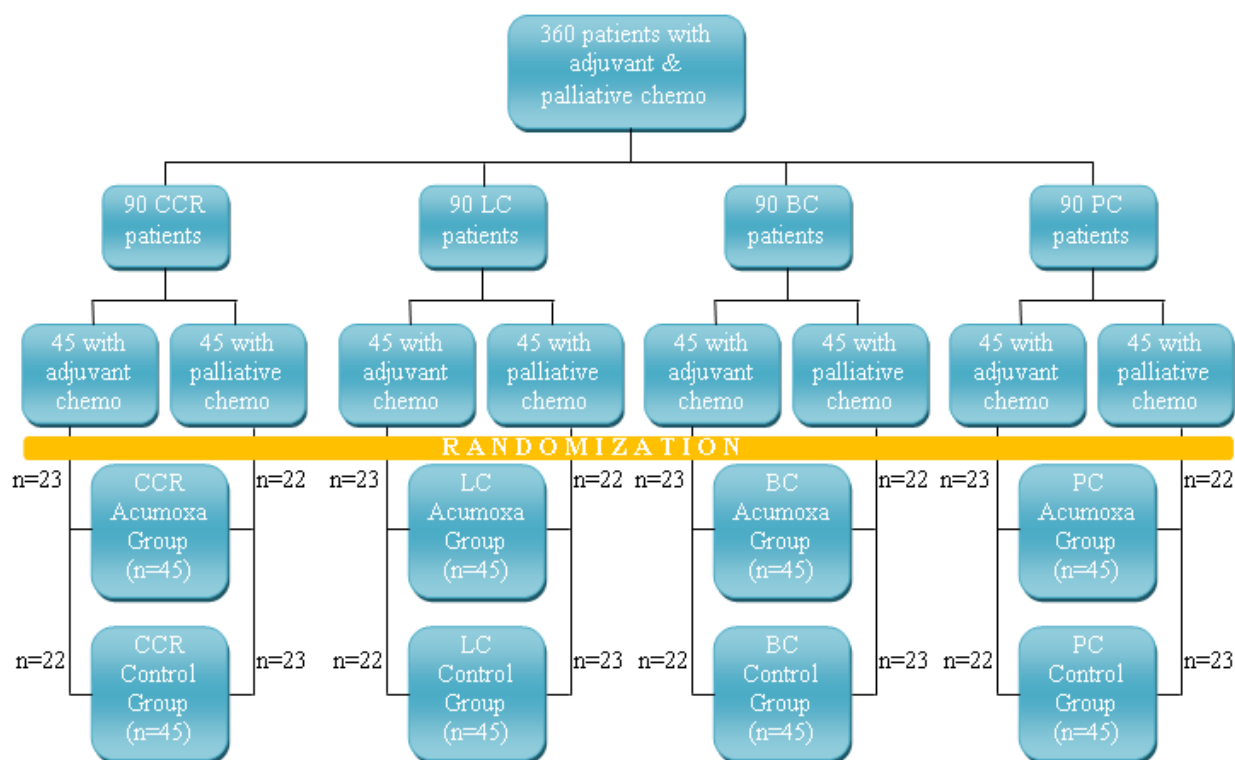
According to a European survey, over a third of cancer patients use complementary therapies to manage cancer symptoms and treatments side effects [1]. Among these therapies acupuncture is the most commonly used by cancer patients in order to manage the symptoms as well as the side effects of treatments [2].

Recent clinical trials have been conducted in order to evaluate the safety, efficacy and effectiveness of acupuncture in managing conventional cancer treatments side effects [3-7]. However, a review on acupuncture clinical trials methodology on oncology patients pointed out several limitations such as the lack of uniformity with the respect to the study population, small sample size, detailed specifications about treatments type and

duration, method of administration and outcome measures[8]. Most of acupuncture clinical trials on cancer patients are focused on a single or a cluster of cancer symptoms and/or cancer treatments side effects and each research group selects the acupoints according to each symptom to treat. These types of studies prevent the establishment of acupuncture protocols standardization that is required for acupuncture clinical trials validity.

Therefore, a new approach is necessary, one that is not focused on symptoms but instead on the global or systemic effect of acupuncture in restoring the normal body functions. This new proposed approach has four concepts as foundation:

First: the side effect that is common to all cancer patients undergoing chemotherapy and



**Figure 1.** Patients groups allocation and randomization. CCR, colorectal cancer; LC, lung cancer; BC, breast cancer; PC, prostate cancer.

probably the root for almost all secondary side effects is the hypofunctional state (Yang deficiency) caused by chemotherapy itself. Chemotherapeutic agents can be considered as a form of *cold-toxic cold* - which leads to symptoms such as coldness, tiredness and stiff limbs, that can be interpreted by the *Shang Han Lun* theory (SHL) [9]. There are two aspects that need to be considered: one, is that an hypofunctional state (*Yang* deficiency) leads to ice coldness, weakness and extreme sensitivity to external cold. The other is that chemotherapy, considered as *toxic cold*, induces cold directly in the body and may affect all the six levels of *Qi* in the SHL. The symptoms presented by the patients differ according to the stage of invasion.

According to the Five Elements Theory [10], cancer leads to an impairment on Metal-Wood axis, represented by the *Lung* (Lu) and *Liver* (Lv). In the SHL, Lu and Lv corresponds to stage IV and

V, and are coupled to *Spleen* (Sp) and *Pericardium* (Pc), respectively. Cancer patients undergoing chemotherapy seem to be in an hypofunctional state, between stage IV (Lu and Sp) and V (Lv and Pc), having symptoms such as sensitivity to cold, weakness of the limbs, tiredness, feelings of dullness and vulnerability to airways infections. Moreover, due to the interior-exterior connections of the meridians, Stage II and Stage III symptoms may be seen, such as nausea, loss of appetite, oedema, diarrhoea, pain in the abdomen and hot-cold sensations [11].

Second: the innate and adaptive immune systems make a crucial contribution to the anti-tumour effects of conventional chemotherapy-based cancer treatments. The Immune system is influenced by two neurophysiological systems: the autonomic nervous system (ANS) via the release of norepinephrine (NE) and the hypothalamic-pituitary adrenal (HPA) axis,

through the release of corticotropin releasing hormone (CRH) which will act on the pituitary gland causing the release of adrenocorticotrophic hormone (ACTH) into the blood stream, which in turn will act on the adrenal glands causing the release of cortisol into the blood stream [12]. Prolonged activation of HPA axis and sympathetic nervous system (SNS) cause changes in the sensitivity of the adrenergic receptors and the Glucocorticoids (Gcs) receptors on immune cells, activating the cholinergic anti-inflammatory pathway with a compensatory high baseline parasympathetic activity (ACTH). However, this level is not sufficient to overcome the active SNS and HPA axis [13-15].

Third: according to Psychoneuroimmunology theory, psychological and emotional stress triggers several alterations in diverse biological responses, in the HPA axis and in the SNS [12]. The activation of these systems may induce a change in the immune cell traffics and a promotion of the inflammation via multiple neuroendocrine and immune pathway [12], leading to a decreased immunity [12]. In the literature it is described that the chronic activation of HPA and SNS cause a decreased activity in NK cells [12].

Fourth: Several studies showed multiple biological responses related to acupuncture, on the neural, endocrine and immunological regulation. This indicates a relationship between acupuncture with the autonomic nervous system, the release of endogenous opioids and the activation of the hypothalamus-pituitary-adrenal axis [8;16-20]. It has been proposed that acupuncture has immunomodulatory effect by acting on ANS and on HPA axis [17-22].

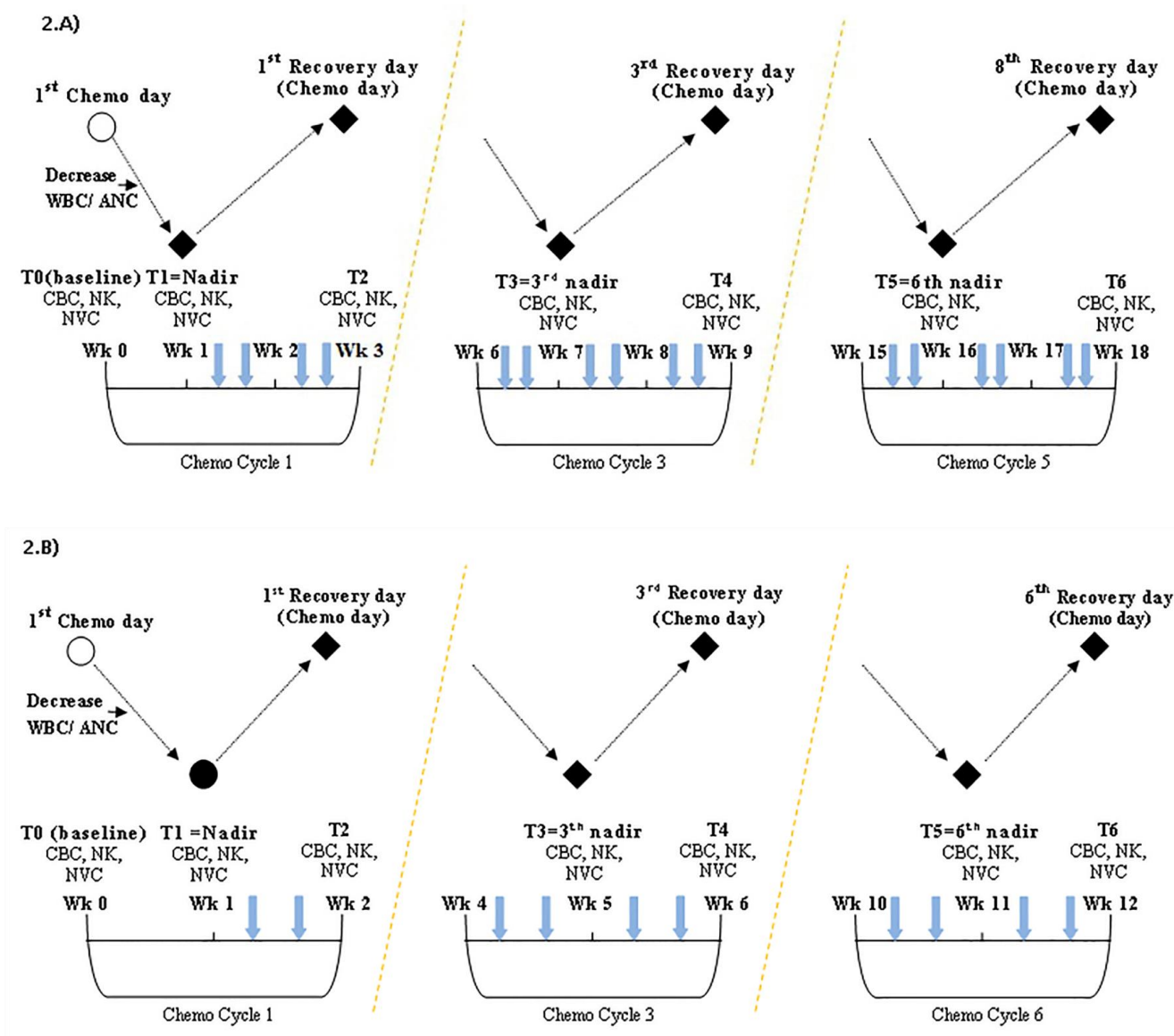
Considering these four concepts as basis for designing acupuncture cancer clinical trials, this will allow not only the establishment of acupuncture standardized protocols but also, the assessment of acupuncture effects in restoring the homeostatic balance between SNS and PNS as well as in restoring the normal functions of HPA axis. Consequently, it will be

possible to correlate such effects on ANS and HPA with symptoms related to cancer conventional treatments as well as patients' immunity status, psychoemotional status, and QOL. These kind of approach will also allow the assessment of the mechanism of action in which single or multiple symptoms can be improved.

From our experience, it was suggested that a standardized acupuncture protocol based on these approach could be beneficial for both colorectal (CRC) and breast cancer (BC) patients undergoing chemotherapy. Both studies assessed acupuncture effects on patients' immune system by measurement of white blood cells, absolute neutrophils, lymphocytes and particularly on NK cells and then correlated these findings with patients QOL and psychoemotional status. The research on CRC showed that acupuncture and moxibustion were able to improve immunity, improve the psychoemotional status (decrease of anxiety and depression), improve patients QOL and minimize chemotherapy side effects [23].

The BC pilot study (results not published) suggested that the same acupuncture protocol used in CRC trial improved significantly BC patients QOL and psychoemotional status. The effects on immune system were not so consistent as on CRC research, which might be due to the small size and heterogeneous population [24]. The results indicate that the acupuncture protocol used is feasible and safe for cancer patients undergoing chemotherapy.

We recently designed a new clinical trial aiming to confirm if cancer patients have a HPA axis and SNS overactivation and whether acupuncture will be able to restore the normal functions of HPA axis as well as to restore the homeostatic balance between the SNS and PNS. The objectives of this future study are to characterize the immune and neurovegetative status of cancer patients undergoing chemotherapy as well as the anxiety and depression levels of these patients; characterize patients' quality of life; explore a relationship between socio-demographic and clinical characteristics, anxiety and depression; explore



**Figure 2. A)** Study flow chart for 21 day-cycle chemotherapy. **B)** Study flow chart for 15 day-cycle chemotherapy. Open circle: baseline sampling before first chemotherapy day (T0). Black solid circle: 1st nadir baseline sampling. Black diamonds: the primary endpoints of the study. Black dashed lines: the expected changes, during chemotherapy, of white blood cells (WBC) and absolute neutrophils counts (ANC). Short, blue down arrows: acupuncture treatments. CBC, complete blood counts; NK, NK cells and subsets (NK<sup>dim</sup> and NK<sup>bright</sup>), NVC, neurovegetative components; Wk, week. T0, baseline time point, T1, 1st chemotherapy nadir baseline; T3, T5, following nadirs; T2, T4, T6, recovery days;. Orange dashed line: interval time line, in which patients continue to receive two weekly AcuMoxa treatments.

the relationship between anxiety and depression levels and the neuroimmunovegetative status; explore the effects of acupuncture in the HPA axis, SNS and PNS and correlate with patients immunity,

psychoemotional status and QOL; explore a relationship between neuroimmunovegetative status, socio-demographic, clinical characteristics and psychoemotional status; and, validate a standardized acupuncture

protocol for cancer patients undergoing chemotherapy.

**Patients and methods.** In Portugal, lung cancer, colon cancer and breast cancer in female and lung cancer, colon cancer and prostate cancer in male counts with the higher mortality rates [25]. Patients will be selected from two Oporto hospital centres after approval by the respective Ethics Committee. Written informed consent will be obtained from all patients before study enrolment. Patients will be eligible for inclusion as follows: *Inclusion criteria*: recently diagnosed or recurrent colorectal cancer, lung cancer, prostate cancer and breast cancer, receiving adjuvant or palliative chemotherapy; no regular use of acupuncture within 120 days prior to enrolment; ability to give informed consent; >18 years of age; *Exclusion criteria*: absolute neutrophils count (ANC) less than 500/ $\mu$ L, (b) platelet count less than 25,000/ $\mu$ L, (c) altered mental state (major psychological diseases), (d) clinically significant cardiac arrhythmias, (e) other unstable medical condition and (f) use of hematopoietic growth factors.

Sample dimension and Allocation. It will be recruited 360 cancer patients in total: 90 patients with CRC, 90 patients with lung cancer (LC), 90 patients with prostate cancer (PC) and 90 patients with BC. Patients for the different type of tumour groups and chemotherapy will be allocated randomly in two groups: AcuMoxa group (n=45) and control group (n=45) (Figure 1). All of the study patients will not be blinded to randomization assignments. Based on previous results [23], sample dimension was calculated using a sample size calculator (<http://clincalc.com/Stats/SampleSize.aspx>), with Alpha=0.05, Beta= 0.1 and power=0.9. The minimum number of subjects needed to be enrolled in the study are 21 in each group (<http://www.ncss.com/software/pass/>).

Study design. It was planned two studies designs according to chemotherapy regimens: a 21-day cycle chemotherapy regimen (BC, PC and LC) (Figure 2.A) and a 15-day cycle chemotherapy regimen (CRC) (Figure 2.B).

Biological samples (peripheral blood, saliva and urine samples) will be collected to all patients enrolled in the study at different time points: Baseline (T0), first chemotherapy nadir (T1), first recovery day (T2), third chemotherapy nadir (T3), third recovery day (T4), sixth chemotherapy nadir (T5) and sixth recovery day (T6) (Figure 2.A and 2.B).

“Baseline”, pre-intervention phase. All patients will be enrolled into a pre-intervention phase. In this phase, the study staff will guide the patient through the consent process in which patients will be informed about the study design, including the use of penetrating needles, and the possible risks of acupuncture treatment (hematoma). Once written consent is obtained, an appointment is made to collect biographic, medical data and *Hospital Anxiety and Depression Scale* (HADS) (26) as well as QOL questionnaires. The biological samples will be collected firstly before the first chemotherapy treatment (T0) and at first nadir (T1) - baseline data.

“Intervention phase”: Patients in the experimental group will receive sessions of acupuncture, twice a week beginning after 1st nadir blood sampling collection [27,28], until the end of chemotherapy cycles (Figure 2). At the end of the intervention, patients in the control group will be offered the acupuncture protocol immediately after they completed blood sampling, as a courtesy. Peripheral blood samples will be collected on the same conditions for all groups: at chemotherapy nadir days (T1, T3, T5) and at the recovery days (T2, T4, T6) (Figure 1.A e B)). At the last day of treatment will be collected QOL and HADS questionnaires.

Acupuncture protocol. The acupoints to be used are at right side LI4, TB5, SP9, LV8 and at the left side PC6, Lu7, ST36 and GB34. Retention needles for 30 minutes. Smokeless moxibustion will be performed at the following points: SI6, TB5, ST32, and CV6; 2 minutes per point. It will be used disposable acupuncture needles with a size of 36G, 0.20× 25mm (Tewa). The de Qi sensation is required [29].

"Follow-up phase": All patients will be monitored for survival at least every 6 months after termination of study treatment(s) during five years. Progression-free survival (PFS), time from randomization to disease progression or death, will include measures such as WBC, serological tumor markers (Carbohydrate antigen (CA) 19.9 for CCR, CA 15.3 for BC, prostate specific antigen (PSA) for PR and Neuron-specific enolase (NSE) for LC) [30], radiographic progression-free survival; progression of soft-tissue disease according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1[31], and QOL questionnaires.

#### Primary Outcomes.

- *Complete blood cell counts (CBC), including NK cells counts by flow cytometry* [23].
- QOL. It will be used "European Organization for Research and Treatment of Cancer Quality of Life Questionnaire" (EORTC QLQ-C30) and its modules for breast cancer (QLQ-BR23), prostate cancer (EORTC QLQ-PR25), lung cancer (EORTC QLQ-LC13) and colorectal cancer (EORTC QLQ-CR29), Portuguese validated versions [32-36].

#### Secondary Outcomes

- *Neurovegetative status.* Levels of circulating *catecholamines and neurotransmitters* (ACTH, NE, E,  $\beta$ -endorphine) by high performance liquid chromatography with electrochemical detection (HPLC-ED) in the urine and serum of all patients Urine samples and peripheral blood from cancer patients will be collected to urine container and EDTA tubes, respectively, in the morning, between 8h and 10h, after they rest for 20 minutes in supine position. *Cortisol levels.* Cortisol levels will be measured in saliva of cancer patients, being collected between 8.00 and 10.00 a.m.

- *Immunity status.* Cells counts and expression of cell-surface markers (T, B, Nk cells, NK<sup>dim</sup> and NK<sup>bright</sup> cells) will be determined by flow cytometry, from patients' peripheral blood. The phenotype of the culture NK cells (NK<sup>dim</sup>/NK<sup>bright</sup> cells) will be performed through the use of the following antibodies: anti-CD56-APC/anti-CD16-PE/anti-KIR-FITC and anti-CD56-APC/anti-CD16-PE/anti-NKG2a-FITC. Evaluation of NK cells functions (production of IFN- $\gamma$  and lyses of target cells after activation) will be performed using peripheral blood NK cells of the patients. The number of viable target cells will be determine by a Flow-cytometry-based NK cytotoxicity assay and the IFN- $\gamma$  production of NK cells during coculture of effector and target cells will be determined in the corresponding supernatants using an ELISA assay [37].
- *Psychoemotional status.* It will be used the HADS, Portuguese version validated by Pais-Ribeiro *et al* [26].
- PFS.

**Statistical analysis.** Statistical analysis will be performed using SPSS version 22 for Windows. The characterization of the sample at baseline will be carried out using descriptive statistics. The results will be statistically analyzed between groups by ANOVA one factor. Where there are differences between AcuMoxa and control groups, the magnitude of these differences will be analyzed using the Tukey test. The intragroup analysis ANOVA for repeated measures and the Tukey test for multiple. All values with  $p < 0.05$  are considered statistically significant. Statistical analysis will be performed by a professional in this area. PFS curves will be computed according to the Kaplan-Meier method; differences in PFS will be compared using the log-rank test comparisons.

**Results.** The study population will be characterized on the basis of socio-demographic and clinical variables as well as the neuroimmunovegetative and psychoemotional status. Analysis on the effect of acupuncture in the HPA axis, SNS and PNS will be performed and correlated with:

- patients immunity (NK cells, total counts and activity),
- patients psychoemotional status and QOL,
- socio-demographic characteristics,
- clinical characteristics (type of cancer, type of treatment, tumor staging), and
- PFS.

Through this approach, it is expected to evaluate the effects of a specific acupuncture protocol on the immune system, more specifically the NK cells and correlate those effects with: a) leukopenia and neutropenia rates, b) chemotherapy-related symptoms; c) psychoemotional status and d) patients' QOL and survival. It is also expected to unveil if a standardized acupuncture protocol is able to restore the homeostatic balance of the HPA axis and ANS and consequently correlate those findings with immunity status, psychoemotional status, QOL and survival as well as with tumor type and staging.

We also expect that this type of research, focused primarily on patients' immunity status, may allow the overcome of the majority of limitations that have been pointed out to acupuncture trials such as small sample size and lack of uniformity regarding the outcome measurements, That is why, in my opinion, it is necessary that different research groups met together allowing to conduct larger and multicentre clinical trials to consistently evaluate the effects of acupuncture in

immunity, QOL and prognosis of cancer patients undergoing chemotherapy.

#### **Abbreviations List:**

ACTH	adrenocorticotrophic hormone
ANC	absolute neutrophils counts
ANC	absolute neutrophils counts
ANS	autonomic nervous system
BC	Breast cancer
CBC	complete blood cell counts
CRC	colorectal cancer
CRH	corticotropin releasing hormone
CV	conception vessel
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
GB	gallbladder
GCs	Glucocorticoids.
HADS	Hospitalar anxiety and depression scale
HPA	hypothalamic-pituitary-adrenal axis
HPLC-ED	high performance liquid chromatography with electrochemical detection
IFN	interferon
LC	lung cancer
Li	Large intestine
Lv	liver
Lu	lung
NE	norepinephrine
NK	natural killer
PC	prostate cancer
PC	pericardium
PFS	progression-free survival
PNS	parasympathetic nervous system
QOL	quality of life
SHL	Shang Han Lun
SI	small intestine
SNS	sympathetic nervous system
SNS	sympathetic nervous system
SP	spleen
ST	stomach
TB	triple burner



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