1 *Astragalus polysaccharide* **(PG2) ameliorates cancer symptom clusters, as well as improves**

2 **quality of life in patients with metastatic disease, through modulation of the inflammatory**

3 **cascade**

4

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11 **Running title:** PG2 mediated broad spectrum of anticancer immunity activities

12

13

14 **Abstract**

15

16 <u>**Background.**</u> Improving patients' quality of life (QoL) is a principal objective of all treatment in

17 any clinical setting, including oncology practice. Cancer-associated inflammation is implicated

18 in disease progression and worsening of patients' QoL. Conventional anticancer therapeutics

19 while selectively eliminating cancerous cells, are evaded by stem cell-like cells, and associated

20 with varying degrees of adverse effects, thus reducing patients' QoL. This necessitates novel

21 therapeutic approaches with enhanced efficacy, minimal or no treatment-related adverse effects,

22 and improved QoL in patients with cancer, especially those with metastatic/advance stage

23 disease. <u>**Methods.**</u> Sequel to our team's previous publication, the present study explores probable

24 effects of *Astragalus polysaccharides* (PG2) on cancer-related inflammatory landscape and

25 known determinants of QoL, as well as the probable link between the two to provide mechanistic

26 insight. In exploratory double blind randomized controlled trial using patients with metastatic

27 disease (n=23), we comparatively evaluated the therapeutic efficacy of high (500mg) or low

28 (250mg) dose PG2 administered intravenously (*i.v.*), with particular focus on its suggested anti-

29 inflammatory function and the probable effect of same on QoL indices at baseline, then at weeks

30 4 and 8 post-PG2 treatment. <u>**Results.**</u> All 23 patients with metastatic disease treated with either

31 low or high PG2 experienced reduced pain, nausea, vomiting and fatigue, as well as better

32 appetite and sleep, culminating in improved global QoL. This was most apparent in the high dose

33 group, with significant co-suppression of pro-inflammatory interleukin (IL)-1β, IL-4, IL-6, IL-13,

1   IL-17, monocytes chemotactic protein (MCP)1, granulocyte-macrophage colony-stimulating

2   factor (GM-CSF), vascular endothelial growth factor (VEGF), tumor growth factor (TGF)-β1,

3   interferon (IFN)-γ, and immune suppressors IL-10, and IL-12. Univariate and multivariate

4   analyses revealed that IL-1β, IL-13 and GM-CSF are independent prognosticators of improved

5   QoL. **Conclusion.** This proof-of-concept study provides premier evidence of functional

6   association between PG2 anti-inflammatory effects and improved QoL in patients with advanced

7   stage cancers, laying the groundwork for future larger cohort blinded controlled trials to establish

8   the efficacy of PG2 as adjuvant anticancer therapy in metastatic or advanced stage clinical

9   settings.

10

11   **Keywords:** Astragalus polysaccharides, PG2, cytokine, inflammatory cascade, cancer cachexia,

12   quality of life, QoL, fatigue

13   **Introduction**

14   Cancer is a complex pathological entity, which is by definition are plurifactorial in

15   etiology, multisymptomatic in character, and binary in prognosis – poor or good. Patients with

16   advanced stage or metastatic cancer are characterized by multiple symptoms on referral to or

17   presentation at medical facilities (1-3). Consistent with accruing evidence, these symptoms, such

18   as pain, disturbed sleep, loss of appetite, fatigue, anorexia nervosa, nausea and vomiting, are

19   seldom orphan symptoms, but rather mostly occur in clusters (1-3). These clusters consisting of

20   ≥ 2 synchronous, often statistically or clinically related symptoms may or may not share same

21   etiology (4). The inter-relatedness of these symptoms, their suggested predictability (1, 2), and

22   influence on the perceived welfare or well-being of patients with cancer (4) brings these

23   symptom clusters to the fore as vital indicators of response to therapy and determinants of

24   patients' quality of life (QoL).

25   Like cancer itself, the patients' QoL is conceptually complex; it is by definition a complex,

26   yet subjective coalescence of the functionality of at least four essential human domains, namely

27   physical, social, occupational, and psychological/emotional (5). These four domains are

28   reflective of the spectrum of evidence-based symptom clusters in advanced or metastatic cancer,

29   namely, cognitive impairment (confusion, agitation, urinary incontinence), neuropsychological

30   (anxiety, depression, and insomnia), fatigue (anorexia, weight loss, and tiredness),

31   gastrointestinal (nausea and vomiting), pain and debility, the presence of which are wholly or in

1 part influenced by the age, gender, primary cancer site, and Eastern Cooperative Oncology

2 Group (ECOG) performance status of patients (6, 7).

3   While the last two decades has been characterized by advances in diagnostic approaches

4 and improved anticancer therapeutic strategies, the synergy between tumor response to

5 therapeutics, progression-free, overall/disease-specific survival, and patients; perception of

6 wellbeing or QoL has become a vital focus of increased medical attention and research in

7 oncology practices, especially in the context of current existent treatment-related adverse drug

8 reactions or events (ADR/Es), with potential or putative therapeutic benefits being outweighed

9 by treatment-related deleterious impact on patients' QoL (8, 9).

10   Concurring with Professor Lesley Fallowfield (5), the balance between drug efficacy and

11 ADR/Es remains relevant and necessitates the discovery of new molecular oncotargets, as well

12 as, design and/or development of novel therapeutic strategies therapies, and QoL measurements

13 in oncology clinics.   The present study was particularly focused on the tetrad –

14 neuropsychological, fatigue, gastrointestinal and pain in patients with cancer. Besides pain,

15 nonspecific cancer symptoms such as fatigue, disturbed sleep, loss of appetite, or anxiety, are not

16 routinely monitored in clinical oncology settings and may not be treated as disease- or treatment-

17 specific toxicities in patients with cancer, subsequently forming a function-limiting or

18 debilitating symptom burden implicated in the causation of non-compliance to chemo- and/or

19 radiotherapy, including dose reduction or withdrawal from treatment, thus, undermining the

20 efficacy of any therapeutic intervention (10).

21   The clustering of cancer symptoms is suggestive of commonality in the underlying

22 developmental mechanisms of the symptom clusters. The synergistic interaction between

23 tumoral immune cells and inflammatory cytokine cascade in the tumor microenvironment

24 culminate in the cancer progression and patients' phenotypic symptom clusters. The systemic

25 predominance of pro-inflammatory cytokines is increasingly being associated with pain, fatigue,

26 cognitive impairment, therapy resistance, worsening of QoL and poor prognosis in patients with

27 advanced or metastatic cancer (11, 12).

28   *Astragalus polysaccharides* (PG2), processed into a sterile powdery form, is the bioactive

29 component of dried a*stragalus membranaceus (Chinese: Huang-Chi)* roots, and elicits a broad

30 spectrum of documented therapeutic effects, including modulation of the immune system; more

31 specifically, PG2 has been shown to induce differentiation of splenic dendritic cells (DCs),

32 expand the $CD11c^{high}CD45RB^{low}$ DC pool, increase the Th1/Th2 ratio, and enhance the T cell-

33 mediated immunity *in vitro* (13*).* Concordantly, there are documentations of the immune-

1 regulating effect of polysaccharopeptide through deregulation of MyD88-mediated signaling

2 pathway (14, 15). However, the probable role of PG2 in the amelioration of cancer symptom

3 clusters and consequent improvement of the QoL in patients with metastatic or advanced stage

4 disease is an evolving theme, and we are learning on-the-go. In fact, while in earlier works, our

5 team provided evidence indicating that higher Karnofsky performance status is predictive of

6 reduced cancer-related fatigue in PG2-treated patients with different cancer types (16), and that

7 PG2 enhances the phenotypic polarization of macrophages, functional maturation of dendritic

8 cells, and T cell-mediated immune responses in patients with non-small cell lung carcinoma

9 (unpublished data), the molecular mechanism underlying these observed and reported findings

10 remains unclear and largely unexplored. In the present study, using a pan-cancer cohort, we

11 explore the effect of varied concentration of PG2 on the neuropsychological, fatigue,

12 gastrointestinal and pain symptom clusters in patients with cancer, and provide evidence that

13 PG2 ameliorates this tetrad of symptom clusters, as well as improves the QoL in patients with

14 metastatic disease, through modulation of the inflammatory cascade.

15

16 **Materials and Methods**

17 *Drugs*

18 250mg and 500mg vials of PG2 lyophilized powder were obtained from PhytoHealth

19 (PhytoHealth Corporation, Taipei, Taiwan), reconstituted in 10mL normal saline solution, and

20 then diluted in 490mL saline solution, *ex tempore.* Rate of i.v. injection was ~ 200mL/h.

21

22 *Patient selection and study design*

23 A cohort of 23 subjects with advanced stage/metastatic cancer randomly selected from our initial

24 multicenter double-blind randomized phase IV study cohort (n = 310) (16). The inclusion and

25 exclusion criteria are as already outlined previously (16). The study was approved by the

26 Institutional Review Board (IRB) of the MacKay Memorial Hospital, consistent with the

27 recommendations of the declaration of Helsinki for biomedical research (Mackay Memorial

28 Hospital approval number: 20160600011 16MMHIS073e) and followed standard institutional

29 protocol for human research. All enrolled patients provided written informed consent and were

30 randomly assigned to either the high (500mg *q.d*) or low (250mg *q.d*) dose arm. PG2 was

31 administered three times per week per cycle of 4 weeks.

1   *Randomization and blinding*

2   Patients were assigned to treatment arms randomly with equal probability using a validated auto

3   randomization system, as previously described (16). Each included patient was assigned a study

4   number with specified treatment plan. The randomization list was only available to the sample

5   manufacturer and was only unblinded after completion of the study.

6

7   *Drug safety evaluation*

8   Drug safety was evaluated as previously described (16). Briefly, the onset of any new PG2-

9   related adverse drug reactions or events (ADR/Es) or exacerbation of existing ADR/Es, were

10   recorded consistent with the Medical Dictionary for Regulatory Activities (MedDRA 9.1). The

11   safety profiles of enrolled subjects included their vital signs and physical examination result.

12

13   *Study endpoints*

14   The primary study endpoint was changes in inflammatory cytokines, including IL-1β, IL-4, IL-6,

15   MCP1/CCL-2, IL-10, IL-12, IL-13, IL-17, GM-CSF/CSF-2, VEGF, TGF-β1, and IFN-γ.

16   Secondary endpoints were QoL scores using the European Organization for the Research and

17   Treatment of Cancer Quality of Life Questionnaire (11 questions (SS11) of the EORTC QLQ-

18   C30) and brief fatigue inventory (BFI). These endpoints were monitored at outpatient visits using

19   blood sampling and questionnaires before and after PG2 injection.

20

21   *Determination of serum inflammatory cytokine levels*

22   The patients' IL-1β, IL-4, IL-6, MCP1, IL-10, IL-12, IL-13, IL-17, GM-CSF, VEGF, TGF-β1,

23   and IFN-γ sera cytokine level were determined using the Cytokine Human Magnetic 30-Plex

24   Panel for the Luminex^{TM} platform (Cat. No. LHC6003M. Thermo Fisher Scientific Inc. Waltham,

25   MA, USA) and Luminex®100/200^{TM} system (Luminex Corporation, Austin, TX, USA). Sera

26   were stored at -80°C until use and processed strictly following the manufacturer's instructions.

27   The quantification criteria were based on fluorescence values of 100 events per region. For

28   cytokine standard curve generation in duplicate, we serially diluted the recombinant standards

29   provided in the kit. Curves were adjusted to a 5-parameter logistic regression model with

30   correlation coefficients (R^2) > 0.95, and the sera cytokine levels (pg/mL) were interpolated from

31   the standard curves.

Statistical analysis

All data are expressed as mean ± standard deviation (SD), 95% confidence interval (95% CI), median/interquartile range (IQR), as appropriate. Comparison between two groups of categorical data was analyzed using the Mann-Whitney *U* test or Pearson's χ2 test, while the significance of differences between time-points was determined using the non-parametric Wilcoxon signed-rank test. For comparison between the two groups of continuous variables, the independent student's *t*-test was used. EORTC and cytokines were analyzed using the log-rank test, Cox's proportional hazards univariate and multivariate regression model. All statistical analyses were performed using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0., Armonk, NY: IBM Corp.). P-value < 0.05 was considered statistically significant.

Results

Patient Characteristics

Of the original 310 subjects screened for eligibility, 23 who apparently met all the inclusion criteria were enrolled for this study and their data analyzed. This present study enrolled 11 from the original 154 in the high dose arm, and 12 from the original 156 in the low dose arm of our PG2 randomized double-blind clinical trial (16). All 23 participants (100%) completed the baseline assessment as well as both primary and secondary endpoint evaluation. All participants had locally advanced or metastatic cancer and were under palliative treatment at the time of PG2 initiation. The general clinico-demographic characteristics of our study cohort, namely age, gender, body mass index (BMI), brief fatigue inventory (BFI) and Karnofsky performance status (KFS) are summarized in **Table 1**.

PG2 elicits significant improvement in patients' QoL

Evaluation of patients' QoL using the EORTC QLQ-C30 SS11 showed that all determinants of QoL including pain, sleep, appetite, nausea and fatigue started to improve even by first visit (visit 1), and were significantly improved by visit 3 after infusion of high or low dose of PG2; however, we find it confounding that while appreciable decreasing trend was observed in vomiting, with no observed or reported case of emesis on visit 3, it was not statistically significant for both treatment arms (low: p = 0.84; high: p = 0.363) (**Table 2**). The observed improvement in QoL is reflects time-dependence and/or pharmaco-cumulative effect of PG2. Interestingly, significant improvement in the global QoL was reported by patients in both low

7

1   dose (p = 0.02) and high dose (p = 0.012) arms of PG2 infusion; this post-PG2 improvement in

2   QoL was both time- and dose-dependent (**Table 2**). With regards to fatigue, the findings of the

3   present study showing that PG2 significantly ameliorates cancer-related fatigue in patients with

4   metastatic or advanced stage disease are consistent at least in part with those of our previous

5   publication (16).

6

7   *PG2 infusion down-modulate patients' pro-inflammatory cytokine profiles*

8   PG2-induced alteration in patients' sera cytokine profile determined using the Cytokine Human

9   Magnetic 30-Plex Panel for the Luminex^{TM} platform and Luminex®100/200^{TM} system revealed

10 that compared to the baseline sera cytokine levels, PG2 induced significant reduction in the

11 production of inflammatory cytokines IL-1β (p = 0.014), IL-4 (p = 0.04), IL-6 (p = 0.012), IL-10

12 (p = 0.019), IL-12 (p = 0.03), IL-13 (p = 0.025), IL-17 (p = 0.018), TGF-β1 (p = 0.024), IFN-γ (p

13 = 0.031), GM-CSF (p = 0.014), VEGF (p = 0.023) and chemokine MCP1 (p = 0.017) in our

14 metastatic disease cohort. This reduction was apparent as early as at the first visit (visit 1) on

15 week 4 of cycle 1 (c1.4) after PG2 initiation, and was time-dependent, however, a strict

16 dependence on dose was not inferable from available data (**Table 3**). It is worth noting that aside

17 the suppression of pro-inflammatory cytokines (IL-1β, IL-4, IL-6, IL-13, IL-17, IFN-γ and other

18 growth factors (TGF-β1, GM-CSF, VEGF) and chemokine (MCP1), PG2 also markedly

19 suppressed anti-inflammatory cytokines IL-10 and IL-12 over the course of our study.

20

21 *PG2-induced improvement in QoL is associated with changes in patients' inflammatory*

22 *cytokine profiles*

23   Cox's proportional hazards univariate and multivariate regression modeling of the EORTC

24 and alteration in patients' sera cytokine levels indicated a functional association between the

25 inflammatory cytokine levels and patients' QoL based on the symptom burden. Drawing from

26 the nine symptom subscales of the EORTC QLQ-C30, namely fatigue, nausea/vomiting, pain,

27 dyspnoea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties s well as the

28 global health/QoL subscale, we established a positive correlation between inflammatory cytokine

29 levels and pain, nausea, vomiting and fatigue, appetite loss and disturbed sleep/insomnia, and

30 poor QoL, as summarized in **Table 4**, whereby for every unit increase in the predictor variable

31 (cytokine level), the outcome variable (EORTC QLQ-C30) increases by the indicated beta

32 coefficient value. These data suggestive of negatively correlated PG2-induced improvement in

33 patients' QoL and changes in the sera level of inflammatory cytokines IL-1β (Pr > |t| = 0.0004),

1   IL-4 (Pr > |t| = 0.0006), IL-6 (Pr > |t| = 0.0002), IL-10 (Pr > |t| = 0.0017), IL-12 (Pr > |t| =

2   0.0041), IL-13 (Pr > |t| = 0.0013), IL-17 (Pr > |t| = 0.001), IFN-γ(p = 0.031)   TGF-β1 (Pr >

3 |t| = 0.0011), GM-CSF (Pr > |t| < 0.001), VEGF (Pr > |t| = 0.0009) and chemokine MCP1 (Pr > |t|

4   = 0.0007) are statistically significant as shown by univariate linear regression. Concordantly, our

5   multivariate linear regression analyses revealed that IL-1β (β = 0.2531, Pr > |t| = 0.0013), IL-13

6   (β = -0.085, Pr > |t| = 0.0442) and GM-CSF (β = 0.1266, Pr > |t| = 0.002) are independent

7   prognosticators of improved QoL (**Table 4**).

8

9

10

11   *PG2, the inflammatory cascade, and the QoL of patients with metastatic cancer*

12   Functional enrichments in the PG2-modulated inflammatory cytokines were analyzed by

13   using the STRINGDB (https://string-db.org) platform for visualization of protein-protein

14   interaction networks. Consistent with our thinking, with an average local clustering

15   coefficient of 0.964, and protein-protein interaction (PPI) enrichment p-value $< 1.0e^{-16}$, our

16   interactome analysis of PG2-modulated IL-1β, IL-4, IL-6, MCP1, IL-10, IL-12, IL-13, IL-17,

17   GM-CSF, VEGF, TGF-β1, and IFN-γ demonstrate that the network formed by these

18   biomarkers exhibited significantly more interactions (n = 63) between each other than

19   expected (n = 12) for a random set of proteins of similar size, thus indicating the cytokines

20   are at least, in part, biologically connected as a group (**Figure 1A**). The concept of a

21   functionally connected group is result of our gene co-expression analysis in human and other

22   organisms (**Figure 1B**). Gene ontology (GO) classification and Kyoto encyclopedia of genes

23   and genomes (KEGG) pathways – based clustering implicated the group in immune response

24   and inflammatory signaling, respectively (data not provided). In addition, hierarchical cluster

25   analysis (HCA) was performed based on the PG2 treatment regimen and changes in our

26   cohort cytokine profile, with the dendrograms defining 2 sample groups (high dose: HC1.1,

27   HC1.4, HC2.1, HC2.4, and low dose: LC1.1, LC1.4, LC2.1, LC2.4), and characterized by a

28   single cluster of our cohort's inflammatory cytokines, namely IL-1β, IL-4, IL-6, MCP1, IL-

29   10, IL-12, IL-13, IL-17, GM-CSF, VEGF, TGF-β1, and IFN-γ. Our cluster distribution

30   analysis showed changes in the inflammatory cytokines profile was statistically significant

31   for HC1.4 (p = 0.013), HC2.1 (p = 0.021), HC2.4 (p < 0.0001), LC1.4 (p = 0.048), LC2.1 (p

= 0.0053), and LC2.4 (p <0.01), with synchronous reduction in expression of all the inflammatory cytokines was observed in both the high and low dose groups (**Figure 1C**).

Discussion

This proof-of-concept study provides premier evidence of the functional association between PG2 anti-inflammatory effects and improved QoL in patients with advanced stage cancers. Our study demonstrated that PG2 concomitantly elicits significant amelioration of the symptom burden or clusters and reduction in the expression level and/or activity of major pro-inflammatory cytokines, including IL-1β, IL-6, IL-12, IFN-γ, and GM-CSF, as early as within a the first month after low or high dose PG2 initiation in patients with metastatic or advanced stage cancer. While our study reveals the expedited therapeutic and QoL-enhancing benefits of PG2 in a clinical setting, we are cautious in the interpretation of our findings to avoid over-interpretation of our data, especially considering the relative small cohort size of the present study and its inherent lack of the conventional treatment (untreated or placebo-treated) control group.

These findings are clinically relevant for medical decision making and selection of therapeutic strategy, especially as cumulative evidence implicates current anticancer therapy (chemotherapy and/or radiation therapy) in occurrence of ADR/Es in patients with cancer undergoing treatment. In fact, though limited, available data does indicate that approximately a tenth of the global population and up to a fifth (20%) of hospitalized patients experience ADR/Es (17, 18), such as depression, loss of appetite, fatigue, disturbed sleep, cognitive difficulties, nausea, vomiting, and pain; and regardless of the unitary or clustered nature of these symptoms, they are associated with exacerbated QoL, with diminished physical, psychological and social functioning. Thus, the present clinical study represents an important progress in providing some mechanistic insight into the occurrence of cancer-related symptom clusters, their inverse association with patients' QoL, and the inflammation-mediated therapeutic activity of PG2.

Regardless of its origin, whether causally-associated with anticancer therapeutics, or inherently characteristic of advanced disease, cancer-related symptom clusters bear adversely on the QoL (5-10) and are independently associated with the survival of patients with metastatic disease (19-21); Therefore, the demonstrated ability of PG2 to ameliorate cancer-related symptom clusters, as well as improve the quality of life in patients with metastatic disease, through modulation of the inflammatory cascade targeting is consistent with progressive

1   therapeutic trends in clinical oncology practice. The clinical use of PG2 as adjunct therapy to

2   target inflammation in patients with metastatic cancer is a therapeutic approach with significant

3   positive implications for the alleviation of patients' symptom burden and enhancement of the

4   clinical efficacy of standard of care anticancer chemotherapy, considering that majority of

5   cancer-related symptom clusters are not only associated with systemic inflammation, but are

6   essentially driven by same, thus, the comeliness of our findings in the context of an highly

7   efficacious symptom cluster-alleviating, QoL-improving, inflammation-limiting, anticancer

8   adjuvant therapy, as proposed by Laird BJ, et al. (22).

9

10   Begging its case for the clinical feasibility and applicability of PG2 as an efficacious

11   anticancer adjuvant/adjunct therapy, the administration of PG2, markedly improved our patients'

12   QoL based on apparent amelioration in 7 out of the 15 QoL domains of EORTC QLQ-C30

13   assessed in this study, namely pain, disturbed sleep, loss of appetite, nausea, vomiting, fatigue,

14   and global QoL, as early as on first visit (visit 1; C1.4), and even more significantly by visit 3

15   (C2.4); this ameliorative effect of PG2 on metastatic disease-inherent or chemotherapy-induced

16   pain, disturbed sleep, loss of appetite, nausea, vomiting, fatigue, and poor global QoL in our

17   metastatic or advanced stage disease cohort was time-dependent and/or pharmaco-cumulative in

18   nature. This is clinically relevant when taken in the context of recent findings by Giesinger JM,

19   et al, showing that patients' QoL is severely impaired in the weeks following the administration

20   of conventional chemotherapy, as demonstrated by increased symptom burden in 9 out of the 15

21   QoL domains of the EORTC QLQ-C30 as early as a week after chemotherapy administration

22   (23).

23

24   Mechanistically, the deregulation of the inflammatory cascade and down-modulation of its

25   associated signals by PG2 with marked amelioration in pain, disturbed sleep, loss of appetite,

26   nausea, vomiting, and fatigue domains of the EORTC QLQ-C30, and resultant QoL

27   improvement is consistent with scientific rationalization and corroborated by another recent

28   work by Laird BJ, et al. on the differential association of QoL in patients with advanced cancer

29   with performance status and systemic inflammatory response; herein they demonstrated that

30   increasing modified Glasgow Prognostic Score (mGPS; systemic inflammation) and

31   deteriorating performance status (PS) are associated with the deterioration in QoL parameters (p

32   < 0.001), indicating that enhanced systemic inflammation is associated with deterioration in

33   patients' QoL parameters independent of PS (24). Thus, we posit that coupled with the immense

1   discomfort, diminished or loss of function, and physical or psychosocial distress inflicted by pain,
2   disturbed sleep, loss of appetite, nausea, emesis, and fatigue on patients with metastatic disease,
3   these adverse determinants of QoL invariably curtail patients' tolerance to anticancer therapy; it
4   thus becomes incumbent on healthcare providers in oncology practices to ensure that these
5   treatment-related and/or advanced disease-inherent cancer symptom clusters are effectively
6   managed to enhance treatment outcome - both subjective (as perceived by the patients) and
7   objective (as confirmed by physical examination, biochemical and instrumental investigations).
8   Thus, as rightly put by Charles Cleeland, "a mechanistic understanding of treatment-related
9   symptoms would be of benefit in drug development, drug evaluation and early integration of
10   appropriate supportive care in treatment planning" (24). Herein, lays the benefit of PG2 as
11   adjuvant therapy; to improve the health status of these patients, while concurrently minimizing
12   function-impairing anticancer treatment-related toxicities, increasing therapy compliance,
13   boosting health-related QoL and potentially conferring some survival advantage.

14

15   The resultant effect of PG2 treatment highlights the therapeutic efficacy and medical
16   validity of inflammatory cascade blockade as an effective therapeutic approach for patients with
17   metastatic or advanced stage disease. Combining results of our present study with those from our
18   teams previously published work (16); PG2 is a potential inflammation-targeting therapeutic
19   option for managing patients with metastatic and advanced disease patients. It is noteworthy that
20   univariate and multivariate analyses suggest a strong positive correlation between EORTC QLQ-
21   C30-based QoL and the responsiveness of the inflammatory cytokines IL-1β, IL-4, IL-6, MCP1,
22   IL-10, IL-12, IL-13, IL-17, GM-CSF, VEGF, TGF-β1, and IFN-γ. Nonetheless, considering
23   possible biases associable with the limitations of the study design which include a histologically-
24   diverse cancer cohort and clinical ethics-based lack of conventional no treatment or placebo
25   control group, we cautiously draw (non-)definitive conclusions based on the significant
26   difference between the baseline and eventual post-PG2 treatment QoL variables of the patients.
27   However, this study lays the foundation for further large cohort prospective randomized clinical
28   studies to clarify if and to what extent the responsiveness of the inflammatory cytokine cascade
29   is a reliable and objective predictor of the QoL for patients with metastatic cancer receiving PG2.

30

31   **Conclusions**

This proof-of-concept clinical study highlights the disease-relevance of changes in systemic levels of inflammatory cytokines, including IL-1β, IL-6, IL-12, GM-CSF, TGF-β1, and IFN-γ as well as the therapeutic efficacy of PG2 as a cancer symptom cluster-alleviating, QoL-improving, inflammation-limiting, adjunct therapy for patients with metastatic or advanced stage cancer. To the best of our knowledge, this is the first study providing evidence that PG2 ameliorates cancer symptom clusters, as well as improves quality of life in patients with metastatic disease, through down-modulation of the inflammatory cascade.

Authors' contributions

WCH, KTK: Project conception, Experimental design, Collation and/or assembly of data, Bioinformatics and computational biology, Data analysis and interpretation, Manuscript writing. OAB, CHW: Project conception, Collation and/or assembly of data, Data analysis and interpretation. KYL, LSY, CTY and JTT: Experimental design, Data analysis, Provision of useful research insight and essential reagents, Administrative oversight. All authors read and approved the final submitted version.

Disclosure Statement

The authors have no competing interests and declare no conflict of interest.

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Tables

Table 1. Cohort Clinico-demographic Characteristics

Variable	Astragalus polysaccharides (PG2) dosage	
	High (500mg)	Low (250mg)
Gender (n,%)		
Male	3(27.27%)	7(58.33%)
Female	8(72.73%)	5(41.67%)
Age (years)		
Mean(SD)	67.27(12.31)	63.67(14.65)
Median(IQR)	64(20)	62(20.5)
95%CI	(59.00-75.54)	(54.36-72.97)
Body mass index (BMI)(kg/m2)		
Mean(SD)	21.22(3.38)	21.08(4.13)
Median(IQR)	21.17(5)	20.67(5.81)
95%CI	(18.95-23.50)	(18.45-23.70)
Baseline BFI score (Cycle 1 visit 1)		
Mean(SD)	8.37(1.18)	7.79(1.67)
Median(IQR)	8.30(2.20)	8.25(2.70)
95%CI	(7.58-9.16)	(6.72-8.87)
Baseline Karnofsky Performance Status score (Cycle 1 visit 1)		
Mean(SD)	70.71(10.44)	65.00(15.08)
Median(IQR)	70(20)	70(25)
95%CI	(63.89-77.93)	(55.42-74.58)

1

*BFI, brief fatigue inventory; SD, standard deviation; IQR, interquartile range; CI, confidence interval

2   **Table 2.** EORTC quality of life questionnaire (QLQ)-C30

Domains	PG2	Baseline	Visit 1	Visit 2	Visit 3	p-value for change
Pain	High	48.48±26.30	26.67±27.89	21.78±22.77	15.00±16.67	0.038
	Low	50.00±36.24	34.44±17.21	23.33±17.89	13.33±33.33	0.014
Sleep	High	78.79±22.47	40.00±43.46	33.33±31.62	8.33±16.67	0.003
	Low	56.94±41.72	44.44±17.21	35.33±34.56	22.22±19.25	0.057
Appetite	High	66.67±29.81	33.33±23.57	30.56±19.48	16.67±19.25	0.013
	Low	68.06±34.42	38.89±25.09	30.00±34.56	22.22±38.49	0.095
Nausea	High	27.27±25.03	13.33±18.26	10.89±19.48	6.89±19.48	0.046
	Low	30.56±28.28	22.22±27.22	16.67±43.46	16.67±43.46	0.093
Vomiting	High	12.12±23.68	7.33±18.26	5.56±13.61	0.00±0	0.363
	Low	11.11±16.41	11.11±17.21	20.00±44.72	0.00±0	0.841
Fatigue	High	86.36±17.98	40.00±14.91	33.33±14.91	8.33±16.67	0.003
	Low	86.11±21.12	33.33±21.08	43.33±32.49	22.22±19.25	0.08
Global QoL	High	30.30±15.49	56.67±25.28	70.83±13.69	83.33±13.69	0.012
	Low	24.31±18.28	61.11±17.21	60.00±25.95	77.78±9.62	0.02
Total score	High	54.82±8.06	39.09±10.62	38.63±8.07	31.06±6.72	0.006
	Low	50.56±9.11	43.68±5.28	44.54±17.78	34.34±6.99	0.004

*EORTC, european organization for the research and treatment of cancer; C30, core 30; QoL, quality of life

3

1   **Table 3.** PG2-induced alteration in patients' inflammatory cytokine profile

Cytokines	Baseline	Visit 1	Visit 2	Visit 3	p-value for change
IL-1β	155.5±44.3	131.8±40.4	119.02±2.15	104.4±3.8	0.014
IL-4	143.1±28.6	132.4±39.9	120.54±4.3	102.8±2.7	0.04
IL-6	147.4±28	133.1±39.4	122.15±1.16	103.6±3.2	0.012
MCP1	143.5±28.3	131.2±40.9	121.63±0.42	103.6±3.3	0.017
IL-10	152.6±45.3	132.4±39.9	122.78±2.06	103.6±3.3	0.019
IL-12	151.1±46.4	133±39.5	122.62±1.82	104.1±2.8	0.03
IL-13	154.3±46.1	132.4±39.9	120.00±3.54	102.8±2.8	0.025
IL-17	153.4±44.9	133.7±38.9	120.23±3.87	102.9±2.7	0.018
GM-CSF	160.3±45.8	133±39.5	119.54±2.88	105.2±3	0.014
VEGF	153.7±44.8	133.6±39	120.23±3.87	105.6±3.6	0.023
TGF-β1	153.3±44.9	132.9±39.5	120.17±3.78	105.1±3.2	0.024
IFN-γ	151.3±46	133±39.5	120.17±3.77	104.7±2.7	0.031

2

3   **Table 4.** Linear regression estimates for EORTC QLQ-C30 with selected cytokines

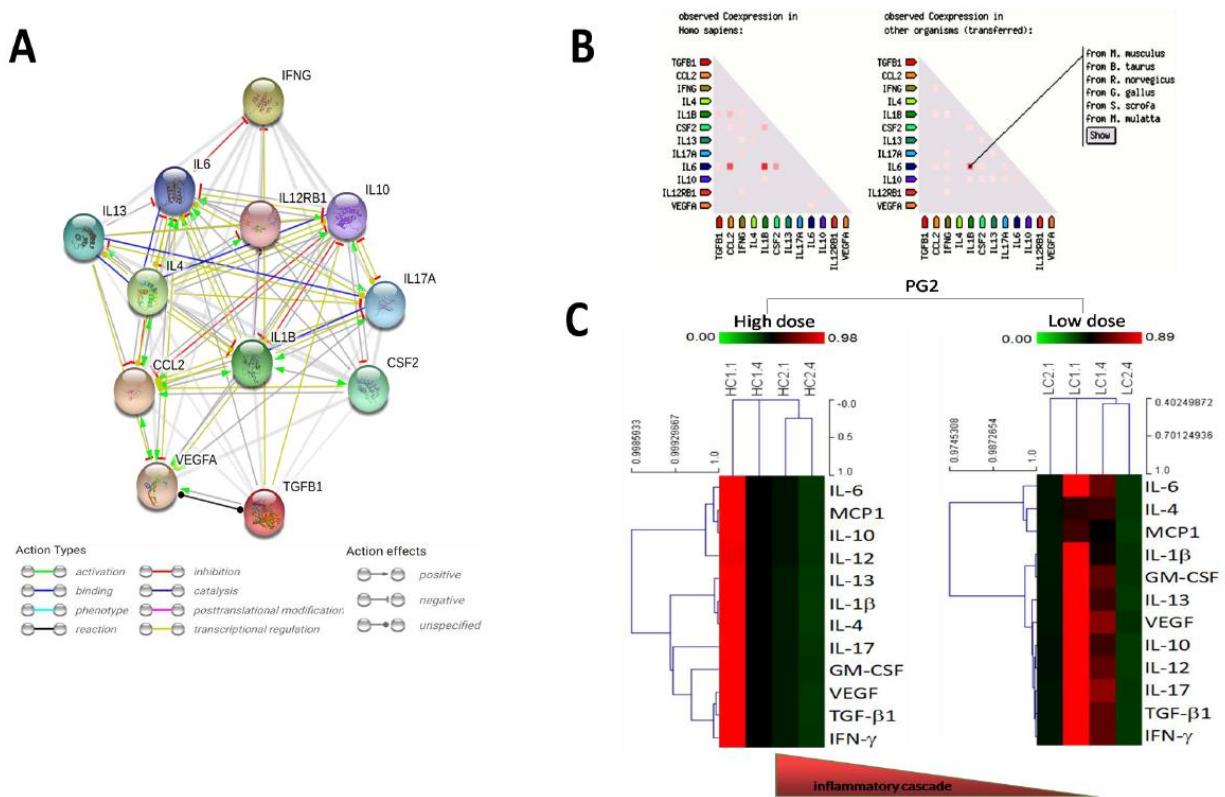| Cytokines	Univariate		Multivariate	
	β	Pr > |t|	β	Pr > |t|
IL-1β	0.043	0.0004	0.2531	0.0013
IL-4	0.051	0.0006		
IL-6	0.051	0.0002		
MCP1	0.046	0.0007		
IL-10	0.037	0.0017		
IL-12	0.034	0.0041		
IL-13	0.04	0.0013	-0.085	0.0442
IL-17	0.041	0.001		
GM-CSF	0.047	<0.001	0.1266	0.002
VEGF	0.041	0.0009		
TGF-β1	0.041	0.0011		
IFN-γ	0.038	0.002		

4

1

2   **Figure 1**

3

4   **Figure 1. PG2-induced improvement in QoL is associated with changes in patients'**
5   **inflammatory cytokine profiles.** (A) Visualization of the protein-protein interaction networks
6   of the inflammatory cytokines of our cohort using STRINGDB platform. (B) Representative data
7   from gene coexpression analysis of the inflammatory cytokines of our cohort. (C) Unsupervised
8   hierarchical cluster heatmap showing the high or low dose PG2-induced changes in expression of
9   our cohort inflammatory cytokines over the study course.