Original Research Article

Effectiveness and Safety of 35 kDa Hyaluronan for Symptom Relief in Advanced Lung Cancer: A Pilot Study Focusing on Pain and Cough

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ABSTRACT

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| **Background:** Advanced lung cancer and its associated pain symptoms place a significant burden on patients. A 35 kDa hyaluronan fragment (HA35) has been confirmed to effectively alleviate inflammatory and neuropathic pain in palliative care settings. This single-arm, prospective pilot study aimed to evaluate the effectiveness of HA35 injection in providing symptomatic relief, particularly for pain and cough, in patients with advanced lung cancer.  **Methods:** This study was a single-arm design without a control group. A total of 12 subjects were recruited from Tuya Amglan Hospital, with 2 subjects excluded; thus, 10 subjects completed the clinical study. Ten patients received 100 mg of HA35 via abdominal deep fat layer injection once daily for 1 week, followed by 100 mg every 3 days for 3 months. All patients were allowed intermittent use of antibiotic compounds and corticosteroids during the treatment period to prevent potential infections.  **Results:** Abdominal deep fat layer injection of HA35 effectively alleviated pain or discomfort (P<.0001) and associated cough symptoms (P<.0001). Chest Computed Tomography (Chest CT) revealed that all lung tumor masses had no significant changes after 3 months of treatment. Interestingly, the treatment significantly improved the fatigue (P<.0001) and facial skin brightness (P<.001) of all patients.  **Conclusion:** HA35 injection may help alleviate pain, discomfort, and cough symptoms in patients with advanced lung cancer. Furthermore, the improvements observed in fatigue and facial skin brightness suggest that HA35 might also contribute to enhancing the mental well-being of cancer patients. |

*Keywords:* *35 kDa hyaluronan fragment; HA35; advanced lung cancer; palliative care; pain; cough; cosmetic therapy*

1. INTRODUCTION

Lung cancer is the most common cancer and the leading cause of cancer-related deaths worldwide [1,2]. The majority of patients have an advanced stage of the disease at diagnosis [3]. In advanced lung cancer, including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), pain and discomfort are the most common symptoms [4]. Approximately 75% of patients experience chronic pain [5,6]. Chronic pain and acute pain may be attributed to the same cause; however, longevity may differ. Chronic pain often persists for more than 12 h with a gradual spread. Effective lung cancer treatment is crucial for relieving patients with advanced lung cancer, reducing the adverse effects of nonsteroidal anti-inflammatory drugs or transdermal opioids, and increasing patient comfort. Importantly, these are also the main objectives of advanced lung cancer palliative care.

Naked mole rats have a higher tissue content of hyaluronan and its fragments. It has been reported that the content of hyaluronan (HA) and its fragments in the tissue of African naked mole rats is as high as 6%. High levels of HA and its fragments make rats insensitive to acid-induced pain, and they do not suffer from cancer while alive [7-9]. Researchers have expressed the high-molecular-weight HA gene in African naked mole rats using a gene transfer method, demonstrating that HA and its fragments could improve the healthspan of mice and make mice insensitive to pain [10]. However, HA (MW>1000 kDa) has a high molecular weight, high viscosity, low solubility in aqueous solution, and poor permeability in human tissues [11-14]. Therefore, for human disease treatment, HA is mainly used for local injection. HA35, known as bioactive HA or B-HA, is a 35 kDa HA fragment that has a bioactive effect on a variety of HA-binding proteins or receptors and can freely cross a filter with a 220 nm pore size [10,15-21]. Particles with a size of <220 nm are usually called nanoparticles (NPs), which are tissue-permeable and particularly common in human venous or parenteral nutrition products. HA35 can be generated by mixing hyaluronidase PH20, also known as SPAM1 (sperm adhesion molecule 1) and injection-grade high-molecular-weight HA at room temperature for 4 h [17], and several clinical studies have shown its efficacy in treating inflammatory and neuropathic pain (registration numbers NCT05756595 and NCT05764226), which is safe for human use [22].

In this study, the clinical efficacy of HA35 or B-HA in the treatment of pain and cough associated with advanced lung cancer was investigated by conducting a short-term self-controlled clinical trial, i.e., an investigator-initiated trial (IIT) (registration number: NCT05852002) [23-24]. At the same time, this is also the first proof of concept clinical study for human body to relieve pain and cough associated with advanced lung cancer.

2. material and methods

**2.1 Materials**

HA35 or B-HA injection samples were obtained from the pharmaceutical company NAKHIA IMPEX, Ulaanbaatar, Mongolia. The recombinant human hyaluronidase PH20 [17] cleaves high-molecular-weight HA (Bloomage Biotech) into the 35 kDa HA fragment HA35 or B-HA (B-HA injection, Registration number L20200708MP07707; Ministry of Health).

**2.2 Patients and study design**

This study was approved by a formally constituted review board (Ethics Committee of Tuya Amglan Hospital), and written informed consent was obtained from all 10 patient volunteers who were recruited by Tuya Amglan Hospital between January and June in 2023 in accordance with the guidelines of the Declaration of Helsinki. In fact, this study recruited 12 patients. 1 patient did not meet the inclusion criteria. Thus, a total of 11 patients was enrolled. 1 patient dropped out during the follow-up. Therefore, 10 patients completed the study protocol. The CONSORT diagram is shown in Fig 1. This study inclusion criteria were as follows: Age ≥18 years (the enrolled patients ranged from 45 to 73 years); Platelet count ≥ (100-300) x109 /L; Patients who were diagnosed with advanced lung cancer accompanied by pain or discomfort and cough symptoms; At least 2 day since completion of chemotherapy or radiation therapy; Must have read, expressed understanding and signed an informed consent document. The exclusion criteria included: a history of other malignancies; pregnancy or lactation in women; coagulation disorders or anemia; and uncontrolled systemic diseases such as diabetes mellitus, hypertension, cardiovascular disease, or chronic kidney disease, which may confound the treatment outcomes.

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**Fig. 1. CONSORT flow diagram**

This single-arm, open-label clinical study investigated the curative effect and safety of 35 kDa hyaluronan fragment injection for the treatment of pain and cough associated with advanced lung cancer and intended to be a proof of concept only. The trial (registration number NCT05852002) was also registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov).

**2.3 Interventions**

HA35 or B-HA (L20200708MP07707) was injected into the abdominal deep fat layer by a registered nurse or a physician (100 mg per day for 1 week) to rapidly alleviate pain, discomfort, and associated cough symptoms. After the first week of treatment, the injection agent was continuously administered at a dose of 100 mg every 3 days for a period of 3 months. Then, pain or discomfort, cough, fatigue, facial skin brightness, and facial expression or spirit associated with advanced lung cancer and local growth of lung cancer masses were assessed using specific methods (described in the 'Primary outcome measures' section). The duration of treatment (days) and adverse events observed following treatment were assessed, and the results were compared with baseline data (before treatment).

**2.4 Outcome measurements**

Primary outcomes were pain or discomfort scores on a 0–10 scale (0=no pain or discomfort, 10=worst possible pain or discomfort) [25,26], cough scores on a 0–10 scale (0=no cough, 10=worst possible cough) [25-27], fatigue scores on a 0–10 scale (0=no fatigue, 10=worst possible fatigue) [28], and facial skin brightness and facial expression or spirit scores on a 0–10 scale (0=worst degree of facial skin brightness, 10=best degree of facial expression or spirit) after treatment. Each of these outcomes was scored as an individual item. The secondary outcome was the result of computed tomography (CT) of the chest after treatment for 3 months (end of the treatment) and the score of modified version Treatment Satisfaction Questionnaire for Medication (TSQM 1.4) after the treatment for 4 weeks and 3 months. The chest CT scans of all 10 patients were compared by clinical radiologists before and 3 months after the start of treatment. High-resolution thin-layer images and volume multiplanar reconstruction scans were used to analyze the structure, size, density, and visual field of the scanned tissue lesion. The relative tumor proliferation rate (T/C%) was calculated as T/C% = TRTV/CRTV × 100, where TRTV is the relative tumor volume at the end of treatment, and CRTV is the relative tumor volume at baseline [29]. The TSQM 1.4 is a generic instrument developed to assess patient satisfaction with therapy [30]. It measures patients’ self-perceived satisfaction with treatment and care via 14 items, including 4 section: effectiveness, side effects, convenience, and global satisfaction [31]. The modified version TSQM 1.4 overall satisfaction scores are calculated for related lung cancer care and pain relieve of the subscales, which range from 0 to10, with higher scores indicating higher patient satisfaction with medication.

**2.5 Adverse events and adverse reactions**

All adverse events (AEs) and adverse reactions were systematically monitored and recorded throughout the study. Investigators documented both spontaneously reported symptoms and clinically observed events at each follow-up visit. Laboratory parameters such as hemoglobin levels and platelet counts were assessed periodically to monitor for signs of anemia. Any adverse events were evaluated for severity, duration, and potential association with HA35 treatment. If any significant or persistent adverse reactions occurred, appropriate clinical measures, including symptomatic management or dose adjustment, were planned in accordance with standard clinical practice.

**2.6 Statistical analysis**

All statistical analyses were conducted using SPSS v.24 (IBM Corp., Armonk, NY, USA) in the present study. Continuous data were evaluated for normality and homogeneity by Student's t-test. Normally distributed data are expressed as the mean (SD) or median (with interquartile range) and were analyzed by one way analysis of variance (ANOVA). Categorical data are presented as percentages. A P value <.05 was considered to indicate statistical significance.

3. results

**3.1 Demographic and clinical characteristics**

A total of 10 patients with advanced lung cancer accompanied by pain or discomfort and cough symptoms were examined at Tuya Amglan Hospital, Ulaanbaatar, Mongolia (Fig 1). The average age of the patients was 67±5 years, the maximum age was 73 years, and the minimum age was 58 years (Table 1). Among them, 7 (70%) patients were male, and 3 (30%) patients were female. All 10 patient volunteers had advanced lung cancer and lung cancer metastasis but no bone metastasis. All of the patient volunteers had varying degrees of pain and discomfort, cough, and fatigue (Table 1). All of the patient volunteers included in this study were allowed to use antibiotic compounds and corticosteroids on and off during treatment to prevent possible infection and reduce pain and discomfort associated with advanced lung cancer.

**Table 1. Demographic and Clinical Characteristics of all Patient Volunteers**

|  |  |
| --- | --- |
| **Characteristic** | **Overall (N=10)** |
| Age (ys) |  |
| Mean (SD) | 67±5 |
| Median (min, max) | 67.5 (56,74) |
| Sex |  |
| Male, n (%) | 7 (70%) |
| Female, n (%) | 3 (30%) |
| Body weight (kg) |  |
| Mean (SD) | 64±11 |
| Lung cancer category |  |
| small cell lung cancer (%) | 2 (20%) |
| non-small cell lung cancer (%) | 8 (80%) |
| Symptom |  |
| Pain or discomfort, n (%) | 10 (100%) |
| Cough, n (%) | 10 (100%) |
| Fatigue, n (%) | 10 (100%) |
| Bone metastasis, n (%) | 0 (0%) |
| Metastasis, n (%) | 10 (100%) |
| Therapy |  |
| Current radiotherapy, n (%) | 0 (0%) |
| Current chemotherapy, n (%) | 0 (0%) |
| Current use of antibiotics n (%)  name and dose | 7 (70%)  sulfamethoxazole and 1.6 g per day |
| Current use of corticosteroids n (%)  name and dose | 7 (70%)  dexamethasone and 0.75 mg per day |

*Notes: values are expressed as n (%), Mean±SD*

**3.2 Primary outcome**

During the study, enrolled patients participated in the study for 3 months and underwent four clinical evaluations at before treatment, after 1 week, 2weeks, and 4 weeks, and the end of treatment. As shown in Table 2, the baseline mean score for pain or discomfort was 4.77±1.82, which was decreased to 0.60±0.31 at the end of treatment (P<.0001). The baseline mean score for cough was 5.50±1.36, which was decreased to 0.82±0.41 at the end of treatment (P<.0001). The baseline mean score for fatigue was 8.35 ±1.07, which was decreased to 1.09 ± 0.65 at the end of treatment (P<.0001). The baseline mean score for facial skin brightness was 1.41±0.72, which was increased to 7.02±0.54 at the end of treatment (P<.001). The baseline mean score for facial expression or spirit was 0.98±0.81, which was increased to 6.82±0.32 at the end of treatment (P<.001). The remission rate scores were both greater than 85%. All differences were statistically significant.

**Table 2. Scores of Outcomes at Baseline (Before Treatment) and at the End of Treatment**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Index** | **Baseline** | **End of treatment** | **Remission rate (%)** | **t** | **P value** |
| Pain or discomfort | 4.77±1.82 | 0.60±0.31 | 87.42 | 6.96 | P<.0001 |
| Cough | 5.50±1.36 | 0.82±0.41 | 85.09 | 10.29 | P<.0001 |
| Fatigue | 8.35 ±1.07 | 1.09 ± 0.65 | 86.95 | 23.08 | P<.0001 |
| Facial skin brightness | 1.41±0.72 | 7.02±0.54 | - | 27.55 | P<.001 |
| Facial expression or spirit | 0.98±0.81 | 6.82±0.32 | - | 23.10 | P<.001 |

*Notes: values are expressed as Mean±SD.*

As shown in Fig 2A and 2B, further analysis revealed that pain and discomfort scores were decreased by 73.58% (1.26±1.18) after 1 week, 79.45% (0.98±0.56) after 2 weeks, and 84.07% (0.76±0.62) after 4 weeks, and the patients showed an improvement from moderate pain to low pain or even painless. Cough scores also decreased by 81.09% (1.04±0.84) after 1 week, 86.18% (0.76±0.75) after 2 weeks and 80.18% (1.09±0.84) after 4 weeks, and the patients progressed from moderate cough to low cough, including no cough. Fatigue symptoms were alleviated after 1 week, and the scores were decreased by 65.75% (2.86±1.35) after 1 week, 60.96% (3.26±1.10) after 2 weeks, and 70.54% (2.46±1.09) after 4 weeks. There was no significant difference between 1 week, 2 weeks and 4 weeks (P＞.05). All patients’ facial skin brightness (P<.001) (Fig 2C) and facial expression or spirit (P<.001) (Fig 2C) were improved gradually after 1–4 weeks, suggesting that HA35 may be a potential cosmetic therapeutic that warrants further clinical investigation.

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**Fig. 2. Advanced lung cancer symptom score and remission rate (%)**

**3.3 Secondary outcome-Chest CT analysis**

According to the results of chest CT, the relative tumor proliferation rate of all patients was ≤43%, which is the minimum level for antitumor activity according to National Cancer Institute standards (Fig 3) [32]. This finding indicated that the lung tumor masses of all patients did not significantly progress at the end of treatment, suggesting that HA35 injection may exert antitumor activity. The modified version TSQM 1.4 overall satisfaction scores after treatment is shown in Table 3. Overall satisfaction increased from 6.60±1.58 to 7.90±1.10 (P<.001). Significant changes in overall scores were observed for relieve of pain, physical functioning, social functioning, and fatigue at the end of treatment. However, short-time treatment also got a high patients satification.

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**Fig. 3. Analysis of the relative tumor proliferation rate with chest CT**

**Table 3. Overall Satisfaction Score after Treatment**

|  |  |  |  |
| --- | --- | --- | --- |
| **Index** | | **Overall satisfaction of treatment for 4 weeks** | **Overall satisfaction of end of treatment** |
| Mean± SD | | 6.60±1.58 | 7.90±1.10 |
| Median | | 7.00 | 8.00 |
| Range | | 4-9 | 6-9 |
| The patients number of score range, n(%) | 0 | 0 (0) | 0 (0) |
| 1-3 | 0 (0) | 0 (0) |
| 4-6 | 4 (40) | 1 (10) |
| 7-9 | 6 (60) | 9 (90) |
| 10 | 0 (0) | 0 (0) |

**3.4 Safety data**

The 35 kDa molecular weight HA35 injection was well tolerated and was safe for all 10 patients. No serious adverse events (SAEs) or treatment-related deaths were reported. There was no significant difference in hemoglobin levels between the treatment follow-up period and before. However, one patient had platelet counts lower than normal number during treatment. But the platelet count returned to normal ranges without discontinuing or reducing the use of HA35 injection. Therefore, it was determined that the decrease was not related to the HA35 injection. Additionally, no gastrointestinal side effects such as nausea or vomiting were observed.

4. Discussion

Lung cancer pain leads to a decrease in the quality of life of patients and imposes an additional psychological burden. The three common causes of pain in patients with advanced lung cancer (https://lungcancer.net/symptoms/pain) are as follows: (1) location of the tumor at the top of the lung and close to several nerves, which accounts for approximately 31% of cases of lung cancer pain; (2) cancer spread to the chest wall, which accounts for approximately 21% of cases of lung cancer pain; (3) lung cancer metastasis to the bone, which accounts for approximately 34% of cases of lung cancer pain [33]. For lung cancer patients with pain, the World Health Organization provides a 3-step approach to relieve pain. Step 1 involves the use of paracetamol or a nonsteroidal anti-inflammatory drug. In step 2, patients are advised to use weak opioids. If pain is not well controlled, the appropriate strong opioids are used in step 3. However, some severe pain may not be satisfactorily controlled in step 3. Therefore, patients are asked to increase the opioid dose over time to relieve pain, which might cause constipation, sedation, nausea, or delirium [33]. Therefore, palliative care with no side-effect is rapidly becoming an important part of the care of advanced lung cancer patients. In this study, we discussed the effectiveness of 35 kDa hyaluronan for the treatment of pain and cough associated with advanced lung cancer.

Abundant high-molecular-weight HA and its fragments have been proven to contribute to cancer resistance and improve lifespan and healthspan [10]. A previously published study showed that HA could modulate pain-regulated TRPV1 channel opening, reducing peripheral nociceptor activity and pain [18]. Because of its high molecular weight, HA has poor tissue penetration ability and is mainly used for local injection for facial reshaping and treating knee osteoarthritis. Nevertheless, our previous study has shown that local injection of the 35 kDa HA fragment (HA35), which has good tissue penetration potential, is effective in treating inflammatory and neuropathic pain (Registration number NCT05756595). It is likely that the 35 kDa HA fragment (HA35 or B-HA) overcomes the poor tissue permeability of high-molecular-weight HA and is capable of entering lung tissue to bind to a variety of HA-binding proteins or receptors, exhibiting bioactivities such as analgesic effects [18]. As well known, common symptoms reported in advanced lung cancer include pain, cough, fatigue, and depression [34]. According to the study of Carol, even in patients who survive more than a year after diagnosis, more than half will report being afflicted with many of these symptoms [34,35]. This study evaluated the effect of HA35 injection (L20200708MP07707) on above index, such as pain and discomfort relief, cough symptoms, and a series of facial [23]. Pain and cough management are important aspects of palliative care for advanced lung cancer patients.

Treatment with 100 mg of HA35 via abdominal deep fat layer injection led to a significant reduction in pain over time. The results indicated that HA35 injection once per day for 1 week effectively alleviated pain or discomfort (Fig 2A, P<.0001) and associated cough symptoms (Fig 2A, P<.0001), suggesting that symptoms associated with lung cancer may be relieved by HA35 in a short time. In most cases, pain was greatly relieved 3 h after the first injection, and cough was significantly relieved in the first 3 days after injection. At the end of treatment, chest CT indicated that none of the lung tumor masses exhibited significant changes. Based on chest CT, the relative tumor proliferation rate was ≤43% in all patients. This finding demonstrated that lung tumor masses did not significantly progress within 3 months of treatment. In other words, HA35 injection might have an inhibitory effect on lung tumor growth. Previous studies [8,10,16,36,37] have reported that high levels of HA and its fragments have anticancer effects, most likely through the regulation of lymphocyte homing [15,17,38]. In fact, the patient perspective is essential to evaluating HA35 injection therapy for the treatment of advanced lung cancer, especially when the treatment objectives are symptom relief. At the end of treatment, we demonstrated that most patients were satisfied with their treatment results.

The findings support the use of HA35 or B-HA (L20200708MP07707) in palliative care therapy for advanced lung cancer [39,40]. HA35 can be safely extracted from human colostrum for human use [22]. In this study, no complaints of any side effects were reported. Interestingly, the treatment significantly improved the fatigue (Fig 2A) of all patients. In most cases, fatigue was significantly relieved after the first week of injection, demonstrating the relief of symptoms associated with lung cancer in a short time following HA35 injection. The results of this study also showed that HA35 significantly improved the facial skin brightness of patients (Fig 2C). These findings indicated that HA35 may be a potential cosmetic therapeutic that warrants further clinical investigation.

However, this study has several limitations. First, it lacked a proper control group and included only a small number of participants. As a proof-of-concept study, it was difficult to obtain timely approval from the ethics committee to include a placebo or negative control group, which would have required the provision of a rescue medication. Second, pain assessment relied solely on the NPRS, which may not comprehensively reflect the multidimensional nature of cancer-related pain. In future clinical trials, a larger cohort will be enrolled, and a placebo or control group will be established to evaluate the optimal dosage and treatment duration of HA35 using multiple assessment tools. Finally, further analysis of clinical data (Table 1) showed that all patients received concurrent treatment with antibiotics and low-dose corticosteroids. Respiratory infections are common in advanced lung cancer and may be aggravated by tumor lesions. The antibiotic sulfamethoxazole and low-dose dexamethasone are commonly used to reduce inflammation and control infection; however, they are often associated with side effects such as nausea and vomiting. In contrast, multiple studies have shown that HA35 provides effective analgesia for inflammatory [41], neuropathic [42], cancer bone metastasis [43], and rheumatoid arthritis-related chronic pain [44]—without causing notable side effects. Therefore, it is possible that the combination of antibiotics and corticosteroids enhanced the therapeutic effect of HA35 while HA35 helped mitigate some of their adverse effects. Given the clinical necessity of using these supportive medications in patients with advanced lung cancer, it is difficult to exclude them entirely from clinical trials. As such, the outcomes observed in this study may result from the synergistic effects of HA35 injections alongside antibiotics and corticosteroids.

5. CONCLUSIONS

HA35 injection may effectively alleviate pain or discomfort and cough symptoms associated with advanced lung cancer. HA35 injection may improve fatigue associated with advanced lung cancer, suggesting its importance in palliative care for patients with advanced lung cancer. HA35 injection may have a cosmetic therapeutic effect on the face, suggesting that HA35 may be a potential cosmetic therapeutic. HA35 injection may have an inhibitory effect on lung tumor growth.

Consent

All authors declare that written informed consent was obtained from the patients for publication of this study. The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Tuya Amglan Hospital (approval number 05/20/2023).

Ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

HA35=B-HA= 35 kDa molecular weight hyaluronan fragment; SCLC= small cell lung cancer; NSCLC= non-small cell lung cancer; HA= hyaluronan; NPs= nanoparticles; PH20= SPAM1= sperm adhesion molecule 1; IIT= investigator-initiated trial; TSQM= Treatment Satisfaction Questionnaire for Medication; ANOVA= analysis of variance.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

References

1 Leiter, A., Veluswamy, R. R., & Wisnivesky, J. P. (2023). The global burden of lung cancer: current status and future trends. Nature Reviews Clinical Oncology, 20, 624–639.

2 Siegel, R., Naishadham, D., & Jemal, A. (2013). Cancer statistics 2013. CA: A Cancer Journal for Clinicians, 63(1), 11–30.

3 Blandin Knight, S., Crosbie, P. A., Balata, H., Chudziak, J., Hussell, T., Dive, C. (2017). Progress and prospects of early detection in lung cancer. Open Biology, 7(9), 170070.

4 Caraceni, A., & Portenoy, R. K. (1999). An international survey of cancer pain characteristics and syndromes. IASP Task Force on Cancer Pain. Pain, 82(32), 263–274.

5 Portenoy, R. K. (2011). Treatment of cancer pain. The Lancet, 377(9784), 2236–2247.

6 Portenoy, R. K., Bruns, D., Shoemaker, B., Shoemaker, S.A. (2010). Breakthrough pain in community-dwelling patients with cancer pain and noncancer pain, part 2: impact on function, mood, and quality of life. Journal of Opioid Management, 6(2), 109–116.

7 Seluanov, A., Gladyshev, V. N., Vijg, J., Gorbunova, V. (2018). Mechanisms of cancer resistance in long-lived mammals. Nature Reviews Cancer, 18, 433–441.

8 Tian, X., Azpurua, J., Hine, C., Vaidya, A., Myakishev-Rempel, M., Ablaeva, J., et al. (2013). High-molecular-mass hyaluronan mediates the cancer resistance of the naked mole rat. Nature, 499(7458), 346-349.

9 Smith, E. S., Omerbašić, D., Lechner, S. G., Anirudhan, G., Lapatsina, L., Lewin, G.R. (2011). The Molecular Basis of Acid Insensitivity in the African Naked Mole-Rat. Science, 334, 1557–1560.

10 Zhang, Z. H., Tian, X., Lu, J. Y., Boit, K., Ablaeva, J., Zakusilo, F.T., et al. (2023). Increased hyaluronan by naked mole-rat Has2 improves healthspan in mice. Nature, 621, 196-205.

11 Snetkov, P., Zakharova, K., Morozkina, S., Olekhnovich, R., Uspenskaya, M. (2020). Hyaluronic Acid: The Influence of Molecular Weight on Structural, Physical, Physico-Chemical, and Degradable Properties of Biopolymer. Polymers (Basel), 12(8), 1800.

12 Zhu, J., Tang, X., Jia, Y., Ho, C. T., & Huang, Q. (2020). Applications and delivery mechanisms of hyaluronic acid used for topical/transdermal delivery - A review. International Journal of Pharmaceutics, 578, 119127.

13 Sander, C., Nielsen, H. M., & Jacobsen, J. (2013). Buccal delivery of metformin: TR146 cell culture model evaluating the use of bioadhesive chitosan discs for drug permeability enhancement. International Journal of Pharmaceutics, 458, 254-261.

14 Park, H. Y., Kweon, D. K., & Kim, J. K. (2023). Molecular weight-dependent hyaluronic acid permeability and tight junction modulation in human buccal TR146 cell monolayers. International Journal of Biological Macromolecules, 227, 182-192.

15 Fraser, J. R. E., Laurent, T. C., & Laurent, U. B. G. (1997). Hyaluronan: its nature, distribution, functions and turnover. Journal of Internal Medicine, 242(1), 27-33.

16 Johnson, L. A., & Jackson, D. G. (2021). Hyaluronan and Its Receptors: Key Mediators of Immune Cell Entry and Trafficking in the Lymphatic System. Cells, 10(8), 2061.

17 Jia, X., Shi, M., Wang, Q., Hui, J., Shofaro, J.H., Erkhembayar, R., et al. (2023). Anti-Inflammatory Effects of the 35 kDa Hyaluronic Acid Fragment (B-HA/HA35). Journal of Inflammation Research, 16, 209-224.

18 Caires, R., Luis, E., Taberner, F. J., Fernandez-Ballester, G., Ferrer-Montiel, A., Balazs, E.A., et al. (2015). Hyaluronan modulates TRPV1 channel opening, reducing peripheral nociceptor activity and pain. Nature Communications, 6, 8095.

19 Hill, D. R., Rho, H. K., Kessler, S. P., Amin, R., Homer, C.R., McDonald, C., et al. (2013). Human milk hyaluronan enhances innate defense of the intestinal epithelium. Journal of Biological Chemistry, 288(40), 29090-29104.

20 Marco, F., Nicola, G., & Gianni, M. (2017). Efficacy and safety of hyaluronic acid (500-730 kDa) ultrasound-guided injections on painful tendinopathies: a prospective, open label, clinical study. Muscles Ligaments Tendons Journal, 7(2), 388-395.

21 Seyed, A. R., Farshad, N., Mahtab, D., Esmaily, H., Ghazihosseini, P. (2020). Ultrasound-Guided Injection of High Molecular Weight Hyaluronic Acid versus Corticosteroid in Management of Plantar Fasciitis: A 24-Week Randomized Clinical Trial. Journal of Pain Research, 13, 109–121.

22 Bellar, A., Kessler, S. P., Obery, D. R., Sangwan, N., Welch, N., Nagy, L.E., et al. (2019). Safety of Hyaluronan 35 in Healthy Human Subjects: A Pilot Study. Nutrients, 11(5), 1135.

23 Konwar, M., Bose, D., Gogtay, N. J., Thatte, U.M. (2018). Investigator-initiated studies: Challenges and solutions. Perspectives in Clinical Research, 9(4), 179-183.

24 Simmons, C. P. L., MacLeod, N., & Laird, B. J. A. (2012). Clinical management of pain in advanced lung cancer. Clinical Medicine Insights: Oncology, 6, 331-346.

25 Cleland, J. A., Childs, J. D., & Whitman, J. M. (2008). Psychometric properties of the Neck Disability Index and Numeric Pain Rating Scale in patients with mechanical neck pain. Archives of Physical Medicine and Rehabilitation, 89(1), 69-74.

26 Vernon, H., & Mior, S. (1991). The Neck Disability Index: a study of reliability and validity. Journal of Manipulative and Physiological Therapeutics, 14(7), 409-415.

27 Harle, A. S., Blackhall, F. H., Smith, J. A., Molassiotis, A. (2012). Understanding cough and its management in lung cancer. Current Opinion in Supportive and Palliative Care, 6(2), 153-162.

28 Donovan, K. A., & Jacobsen, P. B. (2010). The Fatigue Symptom Inventory: a systematic review of its psychometric properties. Supportive Care in Cancer, 19(2), 169-185.

29 Yu, X., Lin, X. J., Wang, S., Liu, X., Li, W., Kou, B.X., et al. (2018). Antitumor Efficacy of Huqizhengxiao (HQZX) Decoction Based on Inhibition of Telomerase Activity in Nude Mice of Hepatocarcinoma Xenograft. Integrative Cancer Therapies, 17(4), 1216-1224.

30 Trask, P. C., Tellefsen, C., Espindle, D., Getter, C., Hsu, M.A. (2008). Psychometric validation of the cancer therapy satisfaction questionnaire. Value in Health, 11(4), 669-679.

31 Liberato, A. C. S., São João, T. M., Jannuzzi, F. F., Landaas, E.J., Wongchareon, K., Rodrigues, R.C.M. (2020). Treatment Satisfaction Questionnaire for Medication (TSQM version 1.4): Ceiling and Floor Effects, Reliability, and Known-Group Validity in Brazilian Outpatients With Hypertension. Value in Health: Regional Issues, 23, 150-156.

32 Park, R., Chang, C. C., Liang, Y. C., Chung, Y., Henry, R.A., Lin, E., et al. (2005). Systemic treatment with tetra-O-methyl nordihydroguaiaretic acid suppresses the growth of human xenograft tumors. Clinical Cancer Research, 11(12), 4601-4609.

33 Simmons, C. P., Macleod, N., & Laird, B. J. (2012). Clinical management of pain in advanced lung cancer. Clinical Medicine Insights: Oncology, 6, 331-346.

34 Tan, I., & Ramchandran, K. (2020). The role of palliative care in the management of patients with lung cancer. Lung Cancer Management, 9(4), LMT39.

35 Tishelman, C., Petersson, L.-M., Degner, L. F., Sprangers, M.A. (2007). Symptom prevalence, intensity, and distress in patients with inoperable lung cancer in relation to time of death. Journal of Clinical Oncology, 25(34), 5381–5389.

36 Seluanov, A., Gladyshev, V.N., Vijg, J., Gorbunova, V. (2018). Mechanisms of cancer resistance in long-lived mammals. Nature Reviews Cancer, 18, 433–441.

37 Smith, E. S. J., Omerbašić, D., Lechner, S.G., Anirudhan, G., Lapatsina, L., Lewin, G.R. (2011). The Molecular Basis of Acid Insensitivity in the African Naked Mole-Rat. Science, 334, 1557–1560.

38 Sackstein, R., Schatton, T., & Barthel, S. R. (2017). T-lymphocyte homing: an underappreciated yet critical hurdle for successful cancer immunotherapy. Laboratory Investigation, 97(6), 669-697.

39 Grond, S., Zech, D., Diefenbach, C., Radbruch, L., Lehmann, K.A. (1996). Assessment of cancer pain: a prospective evaluation in 2266 cancer patients referred to a pain service. Pain, 64(1), 107-114.

40 Chandrasekar, D., Tribett, E., & Ramchandran, K. (2016). Integrated palliative care and oncologic care in non-small cell lung cancer. Current Treatment Options in Oncology, 17(5), 23.

41 Xu, F. H., Treger, D., Ma, X., Jia, X., Shofaro, J. H., Ganbaatar, T., et al. (2024). Local injection of a freshly manufactured 35 kDa hyaluronan fragment reduces neuropathic and inflammatory pain: A clinical study. European Journal of Inflammation, 22.

42 Xu, F., Treger, D., Jia, X., Hui, M., & Ma, Z. (2025). Analgesic and curative effects of 35 kDa hyaluronan fragment on zoster-associated pain: A case report of two patients. *Pain Medicine Case Reports, 9*(1), 51–54.

43 Zhang, Z., Jia, X., Treger, D., & Hui, M. (2024). Low molecular weight 35 kDa hyaluronan fragment HA35 in the treatment of bone metastasis pain: A case report. *Medicine (Baltimore), 103*(31), e39145.

44 Liu, L., Ma, X., Jia, X., Hui, J. H., & Hui, M. (2025). Application of 35 kDa hyaluronic acid fragment in managing persistent localized pain in rheumatoid arthritis: A case report. *Clinical Case Reports, 13*, e70361.