# Palliative treatment efficacy of glucose inhibition combined with chemotherapy for non-small cell lung cancer with widespread bone and brain metastases: A case report

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**Abstract.** Otto Warburg observed in 1924 that cancer cells were dependent exclusively on glycolysis for the production of energy even in the presence of oxygen (the 'Warburg effect'). Consequently, cancer cells require ~19 times more glucose uptake to obtain equivalent amounts of energy as normal cells. The Warburg effect is the scientific basis for positron emission tomography (PET), which has markedly improved cancer detection. During chemotherapy, cancer cells may upregulate their expression of multi-drug resistance proteins and ultimately cause treatment failure. As multi-drug resistance proteins require energy to operate, the present report evaluated the potential clinical efficacy of lowering blood glucose with insulin during chemotherapy for a patient with advanced pulmonary adenocarcinoma with multiple metastases. A 64-year-old male was admitted to the Department of Medical Oncology at Changzhou Tumor Hospital (Changzhou, China) due to an irritating cough and multiple bone pain. PET/computed tomography (CT) with F-18 fluorodeoxy glucose ($^{18}$F-FDG) identified multiple hypermetabolic foci in the right hilum, right upper lung, shoulder blades, thoracic vertebrae, lumbar, sacrum, bilateral iliac crest and pelvis. Additionally, magnetic resonance imaging detected multiple metastases in the brain. The patient received 56 repeat treatments with insulin to induce hypoglycemia combined with reduced doses of chemotherapy over an 8-month period. For each treatment, insulin at 0.2 U/kg body weight was injected intravenously (i.v.), and when blood glucose level reached 2.5-3.0 mmol/l, navelbine (10 mg), cisplatin (10 mg) and fluorouracil (250 mg) were injected (i.v.) over a period of ~10 min. The patient's blood glucose level was returned to normal immediately after chemotherapy with an i.v. injection of 20 ml 50% glucose solution. During the 8-month chemotherapy regimen, the patient received two PET/CT follow-ups. The results demonstrated that the levels of $^{18}$F-FDG uptake in all lesions had been reduced. In addition, the patient exhibited improved appetite and weight gain, a reduced cough, and had less pain. The levels of tumor markers, namely carcinoembryonic antigen, carcinoma antigen 15-3, CYRA21-1, neuron-specific enolase, also declined gradually. These results suggest that controlled, mild hypoglycemia may be safely combined with low dose chemotherapy to provide clinical benefit for advanced non-small cell lung cancer.

## Introduction

Among patients with lung cancer, 30-40% develop bone metastases (1), while 40-50% develop brain metastases (2,3). The median overall survival (OS) rate of these patients with brain metastases is <1 year (3). Current therapeutic approaches for cancer bone and brain metastasis include palliative radiotherapy and systemic therapy with chemotherapy and targeted agents (3,4). Platinum-based combination chemotherapy is the standard of care for non-small cell lung cancer (NSCLC) (5); however, drug resistance limits the therapeutic efficacy of combination chemotherapy in advanced NSCLC (6). Among the key mechanisms by which cancer cell acquire multi-drug resistance is the upregulation of adenosine triphosphate (ATP)-binding cassette (ABC) transporters, including P-glycoprotein (ABCB1), breast cancer resistance protein (ABCG2) and multi-drug resistance associated protein-1, in order to export a number of different chemotherapeutics from the cytoplasm (7). The concept that cancer stem cells may originate from a fusion between an 'altered' pre-malignant cell and a bone marrow-derived stem cell has been proposed (8). Numerous studies have observed higher expression of ABC transporters in cancer stem cells and significant associations of cancer stem cells with multi-drug resistance and tumor relapse (9-12). ABC transporter activity is dependent on ATP (13) and cancer cells produce ATP primarily through glycolysis, even under normal oxygen concentrations, in a process of anaerobic glycolysis known as the 'Warburg effect' (14,15).

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Anaerobic glycolysis produces only two ATPs per glucose molecule and is less efficient than oxidative phosphorylation, which produces ~38 ATPs per glucose molecule (16,17). This suggests that tumor cells require a larger supply of glucose compared with normal cells. Therefore, it was hypothesized that brief and mild induction of hypoglycemia during chemotherapy may increase the sensitivity of cancer cells to chemotherapeutic agents, which may thus bring palliative benefit to patients with advanced cancers by enabling a decrease in the effective dose of chemotherapeutic drugs. The present report describes an advanced pulmonary adenocarcinoma patient with multiple bone and brain metastases who received treatments of insulin-induced hypoglycemia followed by reduced doses of chemotherapy. The study was approved by the Ethics Committee of Changzhou Tumor Hospital (Changzhou, China) and the patient provided written informed consent.

## Case report

A 64-year-old male was referred to the Department of Medical Oncology at Changzhou Tumor Hospital (Changzhou, China) in May 2013 for advanced pulmonary adenocarcinoma with multiple bone metastases. Two months prior, the patient presented with mild coughing and pain in the lower back at a local hospital. During this initial visit, a computed tomography (CT) scan revealed a mass in the upper right lung and mediastinal and right hilar lymph node enlargement. Adenocarcinoma cancer cells were detected by fiber bronchoscope examination and cytological examination. A whole body radionuclide bone scan identified increased radiation uptake in multiple bones, suggesting widespread bone metastases. After 2 cycles of systemic chemotherapy (cisplatin 75 mg/m$^2$ and pemetrexed 500 mg/m$^2$ on day 1 of a 3-week cycle) at the local hospital, the patient experienced no marked relief of the bone pain or irritable cough. The patient was prescribed tramadol (100 mg; PO q12h) to ease the pain. The serum concentration of carcinoembryonic antigen (CEA) increased from 456.7 ng/ml on first admission to >1,000 ng/ml following chemotherapy.

On admission to Changzhou Tumor Hospital, the patient complained of whole body bone pain, an irritating cough, poor appetite, fatigue and substantial weight loss experienced for ~2 months. Prior to chemotherapy, the patient exhibited adequate parameters of organ function, as follows: Adequate bone marrow reserve [white blood cell count, >4,000/ml (reference range, 4,000-10,000/ml), platelet count, >100,000/ml (reference range, 100,000-300,000/ml)]; absence of heart disease and heart rate, 69 bpm [reference range, 60-100 bpm (18)]; serum creatinine, 49.3 $\mu$mol/l (reference range, 45-84 $\mu$mol/l); serum bilirubin and alanine aminotransferase, 7.8 $\mu$mol/l and 15 U/l, respectively (reference ranges, 1.7-23.9 $\mu$mol/l and 13-35 U/l, respectively). References ranges were according to manufacturer's guidelines unless otherwise stated. The baseline levels of serum tumor markers prior to chemotherapy are presented in Table I. For measurement of the serum tumor markers CEA, carcinoma antigen 15-3 (CA 15-3), CYFRA21-1 and neuron-specific enolase (NSE), peripheral blood (2.0 ml) was collected in a vacuum tube (BD Biosciences, Franklin Lakes, NJ, USA) without hematolysis or lipemia from each participant following overnight fasting. Each sample was centrifuged at 1,710 x g for 5 min at 4˚C. The serum was retained following centrifugation, and kept at 4˚C until analysis. The assays were performed within 5 days after samples acquisition. Electrochemiluminescence immunization with an immunology analyzer (Roche Cobas e601; Roche Diagnostics, Basel, Switzerland) was performed to evaluate the tumor markers (19). All tumor marker detection kits were purchased from Roche Diagnostics. According to the manufacturer's recommendations, the reference values of the markers were as follows: CEA, <5.0 ng/ml; CA 15-3, <39 U/ml; CYFRA21-1, <3.3 ng/ml; and NSE, <17.0 ng/ml.

To prepare for chemotherapy combined with glucose inhibition, the patient fasted after 10 pm on the day before chemotherapy to achieve a consistent blood glucose level, though was allowed to drink water. On each day of chemotherapy, the baseline blood glucose levels were tested with a glucometer (SD Codefree; SD BioSensor, Inc., Suwon, South Korea) using standard procedures described by the manufacturer, and the range was determined to be 4.5-5.4 mmol/l [reference range, ≤6.1 mmol/l (20)]. Subsequently, insulin (Nanjing Xinbai Pharmaceutical Co., Ltd., Nanjing, China) at 0.2 U/kg body weight was administered to the patient intravenously (i.v.). After ~20 min, when the blood glucose levels had dropped to 2.5-3.0 mmol/l, chemotherapy was initiated, namely through sequential i.v. injections of navelbine (10 mg; Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, China), cisplatin (10 mg) and fluorouracil (250 mg; both from Qilu Pharmaceutical Co., Ltd., Jinan, China) administered over ~10 min. Following chemotherapy on all treatment days, blood glucose level dropped to 1.6-2.5 mmol/l, and was subsequently elevated to 7-9 mmol/l by i.v. injection with 20 ml 50% glucose. Following each round of treatment, the patient was able to eat a full meal. The chemotherapy treatments were administered 7 times/month for 8 months (56 rounds in total). During each treatment, the patient experienced only mild discomforts of mild heart palpitations and increased sweating. Following the treatments, the patient generally experienced an increase in appetite but no nausea or vomiting. Additionally, the patient's heart rate (69-96 bpm) and blood pressure [90/60-130/88 mmHg; reference range, 90/60-140/90 mmHg (21)] changed little throughout the induced changes in blood glucose levels.

After 3 rounds of chemotherapy, the patient noted a reduction in pain levels. After 2 months, the patient noted an obvious reduction in coughing. After ~4 months, the patient's weight increased from 58 kg (measured on admission) to 63 kg. The serum levels of tumor markers also generally declined gradually (Table I). During the 56 rounds of chemotherapy, the patient presented with grade 1 anemia once following the fifth cycle of chemotherapy, with a serum hemoglobin (Hb) concentration of 110 g/l [reference range of serum Hb concentration in males, 131-172 g/l (22)].

A metabolic response assessment with F-18 fluorodeoxy glucose ($^{18}$F-FDG) positron emission tomography (PET; Infinia vc Hawkeye 4; GE Healthcare, Chicago, IL, USA) (23) and a brain metastases response assessment with magnetic resonance imaging (MRI; Magnetom Trio; Siemens Healthineers, Erlangen, Germany) (24) were performed on the patient. A baseline $^{18}$F-FDG PET/CT scan prior to the start of chemotherapy (Fig. 1) identified increased $^{18}$F-FDG uptake

Table I. Changes in the serum levels of tumor markers during chemotherapy.

| | Tumor markers | | | |
|---|---|---|---|---|
| Chemotherapy cycle | CEA (ng/ml) | CA 15-3 (U/ml) | CYRA21-1 (ng/ml) | NSE (ng/ml) |
| Baseline | >1,000.0 | 159.7 | 165.0 | 35.6 |
| Second | >1,000.0 | 121.8 | 6.5 | 24.0 |
| Third | >1,000.0 | 97.9 | 5.5 | 15.0 |
| Fourth | >1,000.0 | 74.7 | 3.6 | 12.1 |
| Fifth | 941.6 | 50.0 | 2.8 | 9.5 |
| Sixth | 747.5 | 48.0 | 2.5 | 10.1 |
| Seventh | 721.8 | 43.4 | 1.6 | 10.2 |
| Eighth | 395.3 | 41.8 | 1.2 | 10.0 |

Tumor markers were measured prior to each chemotherapy cycle. Data are presented as single measurements. CEA, carcinoembryonic antigen; CA 15-3, carcinoma antigen 15-3; NSE, neuron-specific enolase.

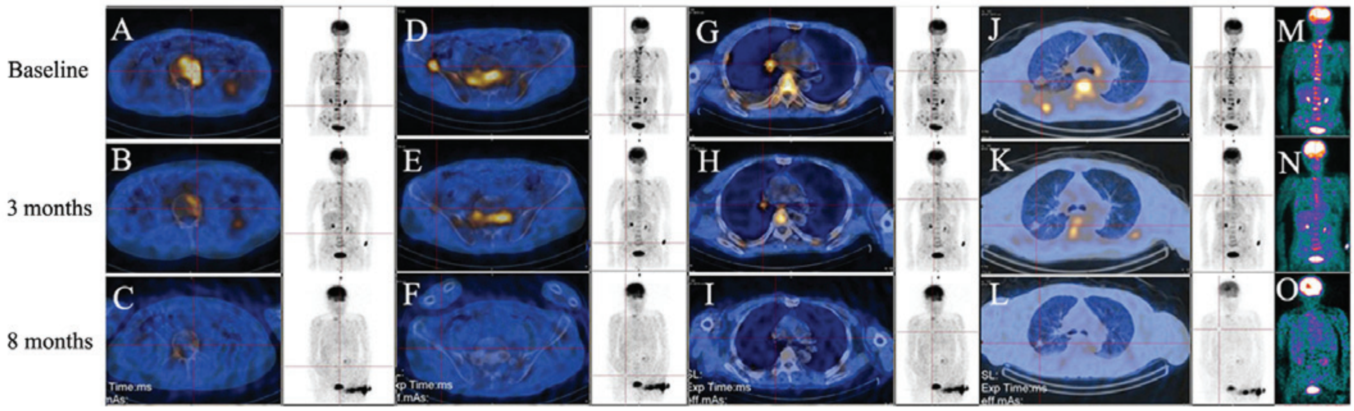


Figure 1. (A-O) $^{18}$F-FDG PET/CT scans. Equivalent full body images on each row are shown. To indicate the changes in $^{18}$F-FDG uptake, areas of lesions (red intersections) with increased $^{18}$F-FDG uptake are displayed. A baseline $^{18}$F-FDG PET/CT scan prior to the start of chemotherapy identified increased $^{18}$F-FDG uptake in multiple bones (A, lumbar; D, right iliac crest; M, colored full body image), in (G) the right hilum and in (J) the right upper lung. Following chemotherapy treatments, $^{18}$F-FDG PET/CT scans identified gradually reduced $^{18}$F-FDG uptake in all the above lesions after 3 months (B, E, H, K and N) and 8 months (C, F, I, L and O) of chemotherapy treatments. $^{18}$F-FDG PET/CT, F-18 fluorodeoxy glucose positron electron tomography/computed tomography.

in the right hilum, right upper lung, shoulder blades, thoracic vertebrae, lumbar, sacrum, bilateral iliac crest and pelvis, and the largest tumor-to-normal (T/N) ratio was 9.1 [normal T/N range, ≤1.0 (23)] in the lumbar, which indicated increased glucose metabolism in the tumor regions. After ~4 months of chemotherapy, $^{18}$F-FDG PET/CT exhibited decreased $^{18}$F-FDG uptake in the same lesions (Fig. 1). $^{18}$F-FDG uptake was further decreased after ~8 months, and the greatest T/N ratio (9.1) had decreased to 1.0, which was paralleled by a reduction in the size of the right upper lung lesion (Fig. 1). Prior to the start of chemotherapy, the head MRI exhibited multiple metastases in the brain, while after 4 months of chemotherapy, MRI identified reductions in the volumes of the brain metastases (data not shown).

At ~2 months after completion of the chemotherapy course, the patient presented with changes in his emotional state, and although the volumes of the brain metastases had been obviously reduced, MRI identified widespread soft meningeal metastases. Testing of bronchoscopy biopsy tissue for epidermal growth factor (EGFR) mutation by direct sequencing (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) indicated that the patient harbored EGFR exon 21 heterozygous mutations (data not shown), and thus was eligible to receive tyrosine kinase inhibitor (TKI) treatment. The patient received whole brain radiotherapy (doses and schedule unknown) followed by TKI treatment (gefitinib, 250 mg/day; AstraZeneca, Cambridge, UK) for ~8 months at the initial local hospital. However, the patient finally succumbed due to meningeal metastasis. The progression-free survival following glucose inhibition combined with chemotherapy was about 11 months, and the OS rate following all treatments was 25 months.

## Discussion

Increased activity of drug efflux pumps, including of the ABC superfamily, is considered to be critical in removing chemotherapeutic agents from cancer cells and causing chemotherapy failure (7). The activity of drug efflux pumps is dependent on ATP hydrolysis, and cancer cells produce ATP primarily through glycolysis, even under normal oxygen concentrations (the Warburg effect) (14,15). Thus, inhibition of tumor glycolysis may impact tumor growth by depleting cellular energy, and may be particularly effective when combined with cancer cell sensitization to therapeutic drugs (25-27). A number of glycolytic inhibitors that target the glycolytic enzymes have been tested, and may be feasible approaches for targeting the limited metabolic behaviors of cancer cells (28-30). Although targeting glucose metabolism seems a viable strategy for cancer therapy, the potential toxic effects of inhibiting metabolic enzymes on normal cells need to be determined in future studies. Distant solid tumor metastases are generally characterized by markedly increased glycolytic activity compared with primary solid tumors, and cancer cells in metastases are typically more resistant to chemotherapy than primary cancer cells (31). In the present lung cancer patient presenting with widespread bone and brain metastases, the $^{18}$F-FDG PET/CT scan identified increased $^{18}$F-FDG uptake in multiple bones and in the primary lung tumor, indicating increased glucose uptake and thus the presence of cancer cells. Meanwhile, the head MRI detected multiple metastases in the brain. It was hypothesized that a brief reduction in blood sugar levels may cause a reduction in ATP production in cancer cells, which in turn may lead to increased sensitivity of the cancer cells to lower dose chemotherapy agents. The present case report first confirmed that a 10-15 min periods of controlled hypoglycemia (1.6-3.0 mmol/l) was safe. During hypoglycemia, the patient experienced only mild heart palpitations and increases in sweating, and when blood glucose levels were returned to normal range with 20 ml 50% glucose solution (i.v.), the patient recovered from the hypoglycemic symptoms. Secondly, administering reduced doses of chemotherapeutics during mild hypoglycemia was an effective treatment method for the patient with advanced lung cancer. The $^{18}$F-FDG PET/CT scans taken every 3-5 months demonstrated that glucose uptake in the tumor lesions was gradually reduced during the chemotherapy course. Notably, PET/CT scan following completion of the chemotherapy course indicated that glucose uptake values in all cancer lesions were almost equivalent to those in adjacent normal tissues. After 4 months of chemotherapy, the MRI identified decreased tumor volumes of the brain metastases. Furthermore, the patient noted pain relief and his cough was alleviated. His appetite also increased, his weight increased from 58 to 63 kg, and nausea and vomiting were absent.

Bone and brain metastases are highly common secondary localizations of disease in patients with lung cancer, and are associated with substantial negative effects on patient quality of life and survival (1,3). The median survival time of patients with these secondary lesions has been reported as 3-7 months (32-34), and the patients frequently require therapeutic intervention. Zoledronic acid is the first and seemingly only bisphosphonate that has exhibited efficacy in the treatment of bone metastases in a randomized phase III trials (35,36). Previous treatments for brain metastases have focused on symptom palliation with whole brain radiotherapy and steroids (3). The TKIs, gefitinib and erlotinib, are also effective options for the treatment of bone and brain metastases, particularly in patients with EGFR mutation (37). Despite advances in the targeted treatment of NSCLC over past years, chemotherapy remains of key importance in the treatment of advanced NSCLC. Platinum-based combination chemotherapy has been a standard of treatment for metastatic NSCLC for more than 30 years, though reaches a therapeutic plateau (3). Therefore, there has been focus on the combination of novel agents with chemotherapy to optimize efficacy, patient survival and overcome acquired resistance (38). It has been proposed that insulin as a pharmacological agent induces a switch from a non-cycling to cycling status in tumor cells (39), thus increasing the uptake of chemotherapeutic agents (40) and their cytotoxic effect in cancer cells (41). In the current patient, insulin-induced hypoglycemia followed by reduced-dose chemotherapy drugs was observed to reduce the glucose uptake and sizes of metastatic tumor lesions, and to improve the patient's quality of life, notably by reducing bone pain and analgesic use, improving appetite and increasing body weight. It may be speculated that hypoglycemia inactivates ABC transporter pump activity, particularly in cancer cells due to the Warburg effect. This may cause increased accumulation of chemotherapeutic agents in cancer cells. However, further experiments are required to verify these mechanisms. From the current treatment study, it may be proposed that insulin-induced hypoglycemia followed by low dose chemotherapy is a viable choice for the palliative care of patients with advanced solid tumors, particularly of those patients who can not tolerate or have failed traditional chemotherapy regimens. Future studies of similar cases are now required to validate the feasibility of this treatment method.

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