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***In-vitro* proliferation assay with recycled ascitic cancer cells in malignant pleural mesothelioma: A case report**

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Author contributions: Anayama T organized the entire study protocol and mainly wrote the manuscript; Taguchi M and Tatenuma T performed the CD-DST; Okada H and Kume M performed the KM-CART; Miyazaki R, Hirohashi K, and Kume M were responsible for the clinical treatment of the patient; Orihashi K was responsible for the entire study.

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Abstract

BACKGROUND

We report the first case, to the best of our knowledge, of massive ascites due to recurrent malignant pleural mesothelioma that was controlled using KM-cell-free and concentrated ascites reinfusion therapy (KM-CART). The tumor cells derived *via* KM-CART were utilized secondarily in an *in vitro* cell growth assay using the collagen gel droplet-embedded culture drug sensitivity test (CD-DST) to investigate anticancer drug susceptibility.

CASE SUMMARY

A 56-year-old man presented with recurrent malignant mesothelioma with massive ascites; more than 4000 mL of ascitic fluid was removed, filtered, and concentrated using KM-CART, and the cell-free ascitic fluid was reinfused into the patient to improve quality of life. Cancer cells isolated secondarily in an *in vitro* proliferation assay using CD-DST exhibited low sensitivity to pemetrexed and high sensitivity to gemcitabine. Treatment with gemcitabine maintained stable disease for 4 mo.

CONCLUSION

The combination of KM-CART and CD-DST may be a promising treatment option for malignant ascites associated with malignant mesothelioma.

Key words: Ascites; Cancer; Malignant; Mesothelioma; Pemetrexed; Palliative therapy; Case report

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Core tip: Massive ascites due to recurrent malignant mesothelioma was controlled with innovative cell-free and concentrated ascites reinfusion therapy, and the derived tumor cells were utilized secondarily in an *in vitro* cell growth assay that contributed to the personalized chemotherapy for the patient.

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INTRODUCTION

Malignant mesothelioma is a rare and insidious neoplasm with a poor prognosis. Pemetrexed-cisplatin (PEM-CDDP) combination therapy was established as a standard treatment with level I evidence and grade A recommendation^[1]. Only patients with favorable prognostic features, histology, and staging are referred for radical treatment involving extensive cytoreductive surgery consideration^[2]. For cure, two radical surgical procedures, extra-pleural pneumonectomy (EPP) and pleurectomy/decortication, are performed in combination with chemotherapy and/or radiotherapy. A systematic review showed that the median overall survival after EPP was 9.4–27.5 mo, with a 5-year survival rate of 0%–24%^[3].

Ascites is a unique form of malignant pleural mesothelioma (MPM) recurrence^[4]. Although puncture and aspiration can improve the symptoms of ascites, repeated procedures result in loss of large amounts of electrolytes and albumin. Cell-free and concentrated ascites reinfusion therapy (CART) was developed as a palliative therapeutic option for controlling massive ascites and improving the patient's quality of life^[5,6]. KM-CART, a novel CART system developed in 2011, is a highly efficient method of processing tumor cell-rich malignant ascites^[7,8]. In this report, we described a case of massive ascites due to recurrent MPM that was controlled using KM-CART. Here, the tumor cells derived *via* KM-CART were utilized secondarily in an *in-vitro* cell growth assay using the collagen gel droplet-embedded culture drug sensitivity test (CD-DST) to investigate anticancer drug susceptibility^[9,10].

CASE PRESENTATION

Chief complaint

A 56-year-old man presented to our hospital with right pleural effusion.

History of present illness

The patient had a long history of outpatient treatment for hypertension. Although he was asymptomatic, a routine chest computed tomography (CT) scan revealed the presence of pleural effusion in the right lung.

History of past illness

He had no prior history of hospitalization, operations, or injuries.

Personal and family history

He had no significant childhood illnesses. His deceased parents had no history of malignancy. He had two healthy siblings. He had a 30-year smoking history of 1 pack/d between the ages of 20 and 56 years (30 pack/year). He was occupationally exposed to asbestos from the age of 24 to 30 years.

Physical examination upon admission

Upon physical examination, his respiratory sounds were slightly attenuated in the right back. His cervical, supraclavicular, and axillary lymph nodes were not palpable.

Laboratory examinations

Preoperative complete blood count and biochemical examination of blood showed almost normal findings. The levels of serum tumor markers such as squamous cell carcinoma related antigen, cytokeratin fraction, and carbohydrate antigen 19-9 were

within normal limits, while elevation of carcinoembryonic antigen level to 10.5 ng/mL (< 5.0 ng/mL) was observed.

Imaging examinations

A chest radiograph and chest CT revealed right pleural effusion, but no tumorous shadow was pointed out in the chest. Cytological examination of the right pleural effusion, sampled *via* thoracentesis, revealed malignant mesothelioma cells. He had been diagnosed with c-T1aN0M0 c-stage IA^[11] malignant pleural mesothelioma.

He underwent right EPP and lymph node dissection with curative intent. Pathological examination of the resected tissue yielded a diagnosis of diffuse MPM, epithelioid type, p-T2N2M0, and p-Stage III. He did not receive postoperative adjuvant chemotherapy. Thirteen months after operation, multiple pulmonary metastases were detected on F-18 fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) (Figure 1). The patient refused CDDP/PEM treatment because of the anticipated side effects of CDDP; he received 8 mo courses of single-agent PEM therapy (500 mg/m²) and achieved stable disease^[12].

Twenty-one months post-surgery, the patient was re-hospitalized because of abdominal pain and tightness, dyspnea, and anorexia due to massive ascites. Accordingly, we diagnosed recurrent mesothelioma presenting with massive malignant ascites.

FINAL DIAGNOSIS

The final diagnosis was recurrent mesothelioma presenting with massive malignant ascites.

TREATMENT

KM-CART was performed as a palliative treatment (Figure 2). An 8-Fr trocar tube was inserted into the Douglas pouch under local anesthesia for ascitic fluid discharge into a dedicated bag. The discharged fluid, with a total volume of 4050 mL, was filtered and concentrated, while raw ascites was drained from the body. The filtrate concentrate, with a fluid volume of 610 mL and protein concentration of 8.0 g/dL, was reinfused intravenously into the patient. After KM-CART therapy, the patient's body weight decreased by 1.7 kg, his abdomen flattened, and the abdominal pain and dyspnea quickly disappeared. He was able to ingest a normal amount of food the following day.

Then the recycled 5.4×10^8 tumor cells were subjected to CD-DST according to a previously reported method^[10-13]. The isolated malignant mesothelioma cells were less susceptible to previously used systemic chemotherapy agent PEM (109.4% growth rate relative to the normal control). The other agents such as CDDP, carboplatin, nedaplatin, paclitaxel, docetaxel, and vinorelbine also exhibited less susceptible. However, the tumor cells were sensitive to gemcitabine (GEM) (growth rate reduction to 21.9% relative to the normal control) (Figure 3). Therefore, we administered two courses of second-line GEM chemotherapy (1000 mg/m² on days 1, 8, and 15).

OUTCOME AND FOLLOW-UP

Although the patient received palliative treatment, he died 2 years and 4 mo after the initial surgery. However, we observed no increase in the amount of ascites following KM-CART, during GEM therapy, or until 4 mo before death. The overall timeline of treatments and outcomes is presented in Figure 4.

DISCUSSION

MPM may spread to various locations. Local recurrence is generally associated with lymph node disease (65%), pleural effusion (64%), chest wall involvement (43%), contralateral lung disease (36%), pericardial infiltration (29%), and pericardial effusion (12%). Patterns of distal spreading are characterized by parenchymal lung metastasis (27%) and peritoneal/omental disease (24%) with malignant ascites in 16% of cases. These conditions are often followed by bone metastasis (20%) and the development of subcutaneous metastatic nodules (19%)^[14].

The patient exhibited recurrent mesothelioma presenting with massive malignant

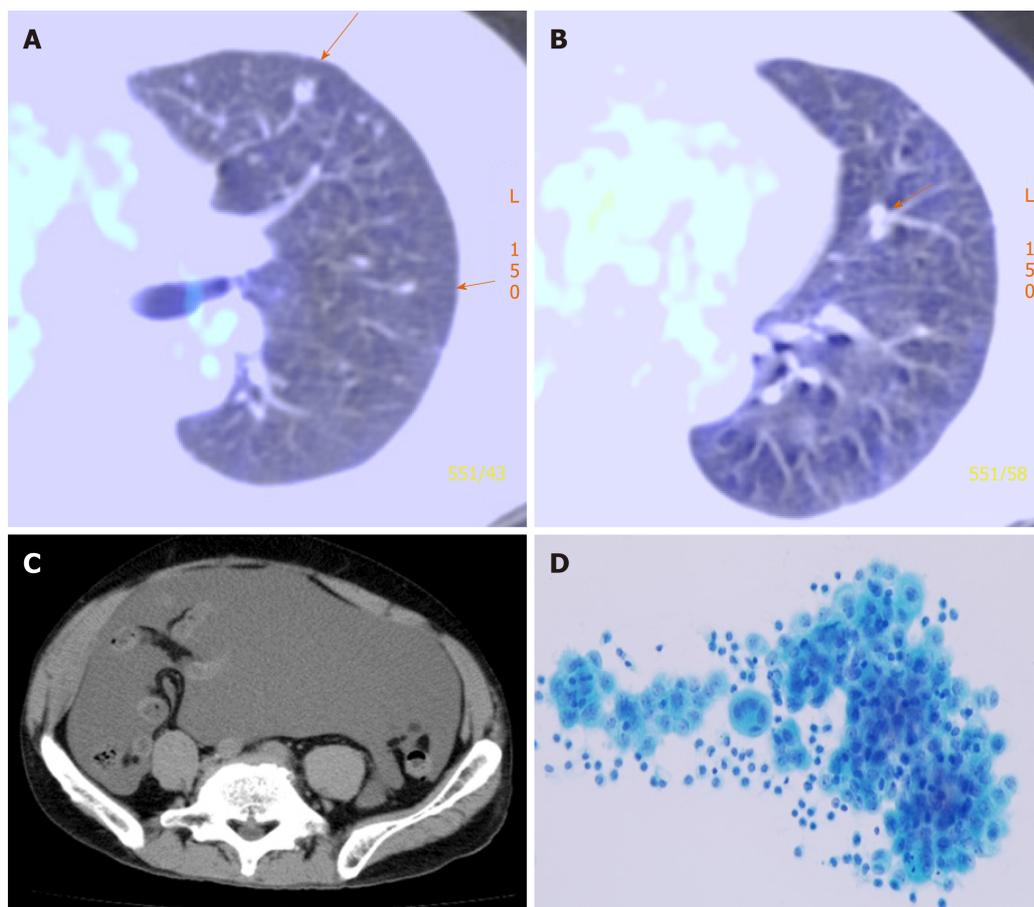


Figure 1 Postoperative recurrence of malignant mesothelioma. A, B: F-18 fluorodeoxyglucose positron emission tomography/computed tomography (CT) showing multiple pulmonary metastases (orange arrows) that occurred 13 mo postoperatively; C: CT showing massive ascites after eight courses of pemetrexed therapy; D: A photomicrograph of mesothelioma cells in the ascitic fluid obtained *via* abdominocentesis.

ascites during systemic chemotherapy with PEM. Generally, the fluid associated with ascites is removed by paracentesis in order to relieve abdominal pain as needed as part of palliative care. KM-CART, a unique approach to CART developed by Dr. Matsusaki *et al*^[6] involves a simple and innovative system consisting of an infusion pump and a suction system. Here, the ascitic fluid is filtered from the outside to the inside of the filtration membrane, in the opposite direction to the hemodialysis system. Therefore, clogging of the filtration membrane can be eliminated by a reverse-direction (*i.e.* inside to outside) bolus flush of physiological saline in the CART system circuit. The flushed cancer cells can then be stored in an isolated bag and utilized for *in vitro* growth assay.

CD-DST is a culture-based anticancer drug susceptibility test that uses fresh tumor tissues obtained from various excised solid tumors that have a high probability of growth in *in vitro* environments. The usefulness of CD-DST has been demonstrated in lung cancer^[15-17], gastric cancer^[18], colorectal cancer^[19,20], and MPM studies^[21]. In our case, the cultured cancer cells were resistant to PEM but highly sensitive to GEM. Therefore, second-line chemotherapy with GEM enabled the patient to maintain stable disease for 4 mo.

This case report had one important limitation. CD-DST is an *in vitro* growth assay that originally utilized highly viable fresh cancer cells excised surgically; malignant mesothelioma cells extracted from ascites may exhibit lower viability than to those extracted from a surgical specimen. It is possible that the low viability may have affected the *in vitro* growth rate success of the assay. We recommend further comparisons to clarify this point.

CONCLUSION

To the best of our knowledge, this is the first report of the use of KM-CART and CD-DST of recycled massive ascitic cancer cells. The combination of these two techniques may provide a unique option for personalized treatment of massive malignant ascites.

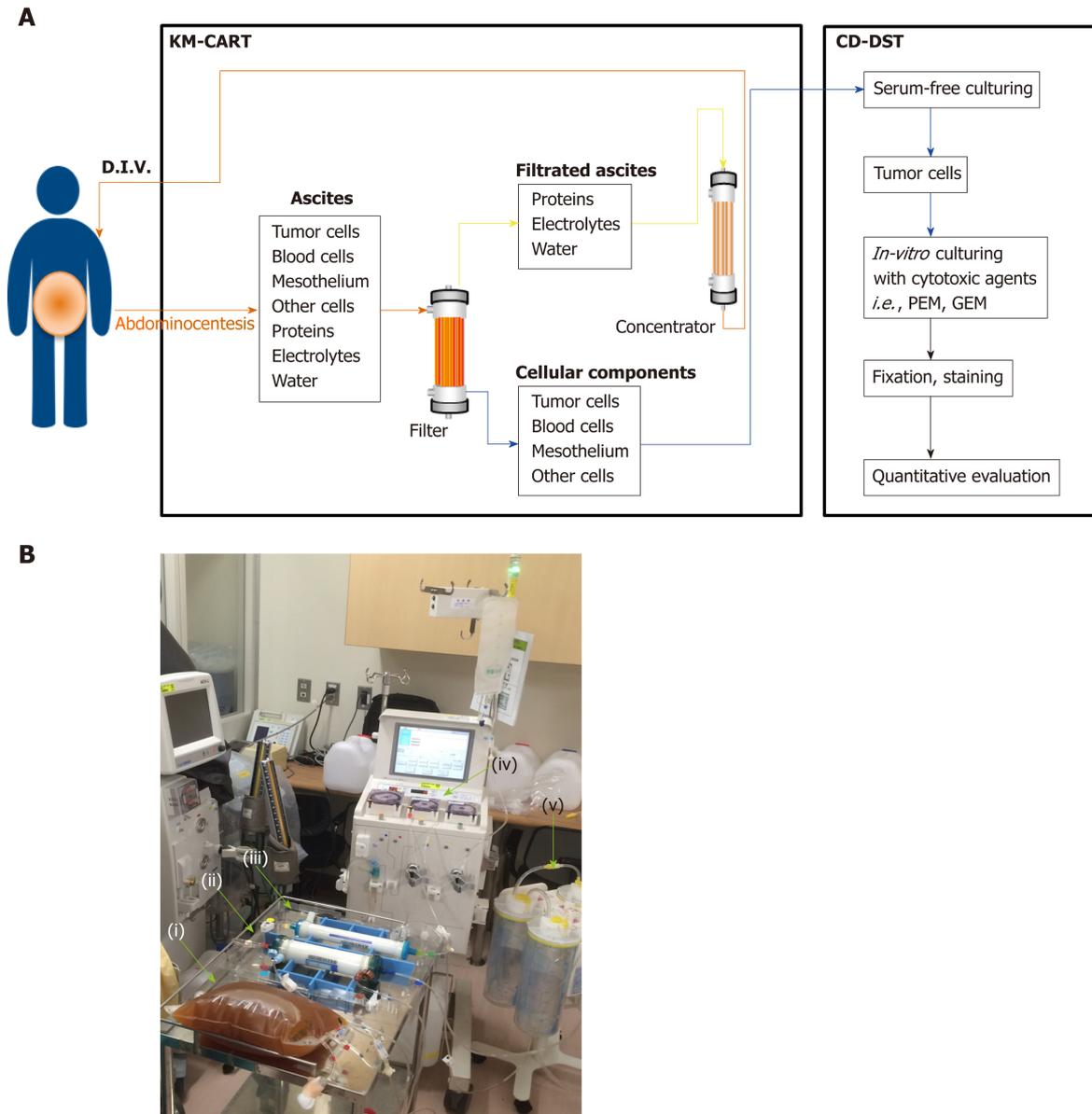


Figure 2 Use of KM-cell-free and concentrated ascites reinfusion therapy and collagen gel droplet-embedded culture drug sensitivity test. A: Schema of KM-cell-free and concentrated ascites reinfusion therapy (KM-CART) and collagen gel droplet-embedded culture drug sensitivity test applied in the presented case; B: Image of the KM-CART system used for actual treatment. Ascitic fluid obtained via abdominocentesis (i) was immediately processed using the KM-CART system consisting of an ascites filter (ii), an ascites concentrator (iii), a roller pump (iv), and an aspirator (v). KM-CART: KM-cell-free and concentrated ascites reinfusion therapy; CD-DST: Collagen gel droplet-embedded culture drug sensitivity test.

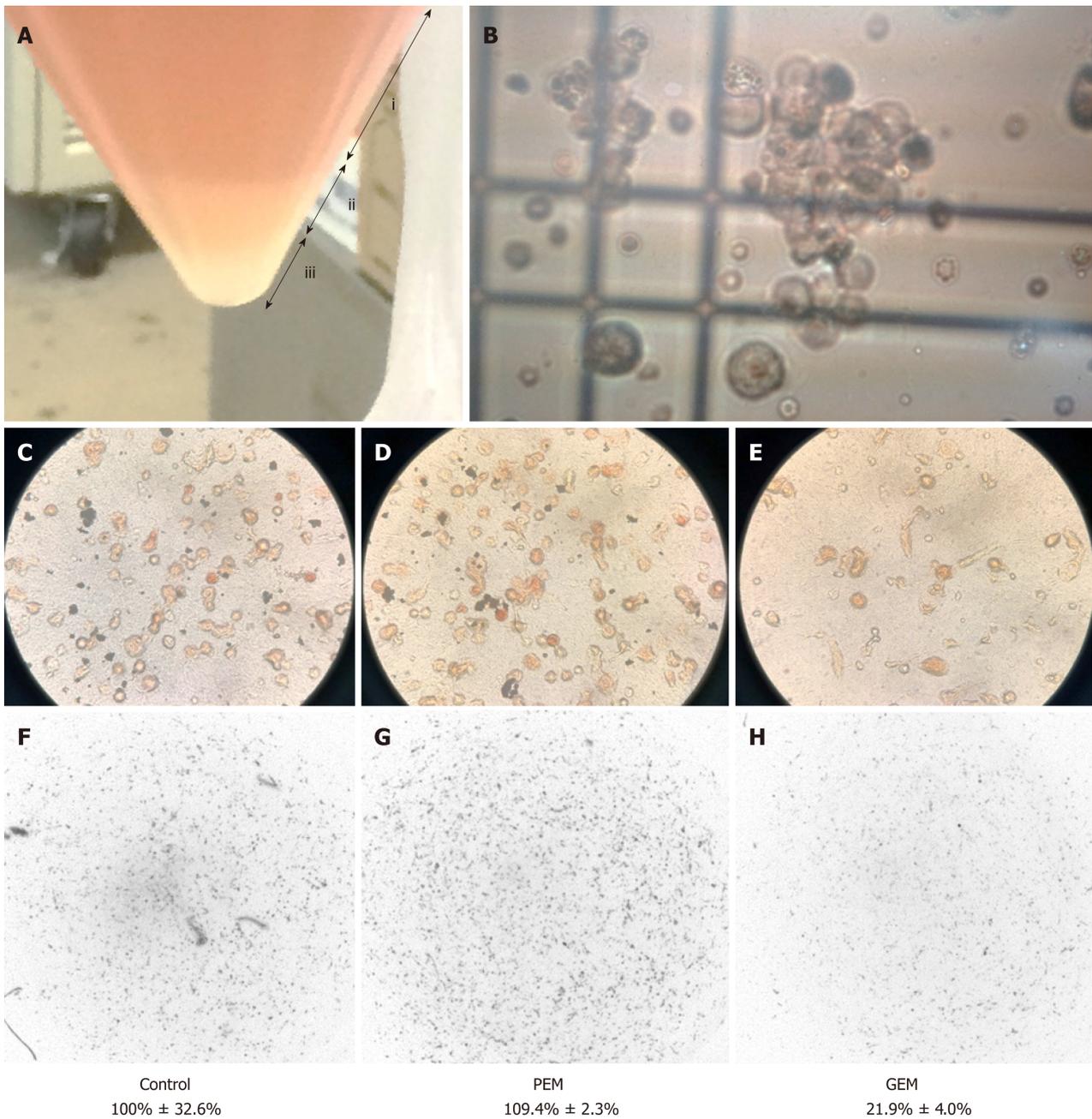


Figure 3 Collagen gel droplet-embedded culture drug sensitivity test assay of mesothelioma cells obtained from ascites. A: The filtered cancer cells were centrifuged to divide the fluid into a supernatant (i), mucus component (ii), and cell pellet including mesothelioma cells (iii); B: After cell counting, the mesothelioma cells were used to conduct the collagen gel droplet-embedded culture drug sensitivity test (CD-DST). Tumor cells were incubated without any cytotoxic drug; C: Or in the presence of cytotoxic drugs such as D: pemetrexed (PEM) and E: gemcitabine. After the proliferation assay, the final number of cancer cells was quantified using a dedicated system; F: Compared to a normal control, PEM did not reduce cell growth, while gemcitabine reduced the tumor cell growth rate to 21.9%. PEM: Pemetrexed; GEM: Gemcitabine.

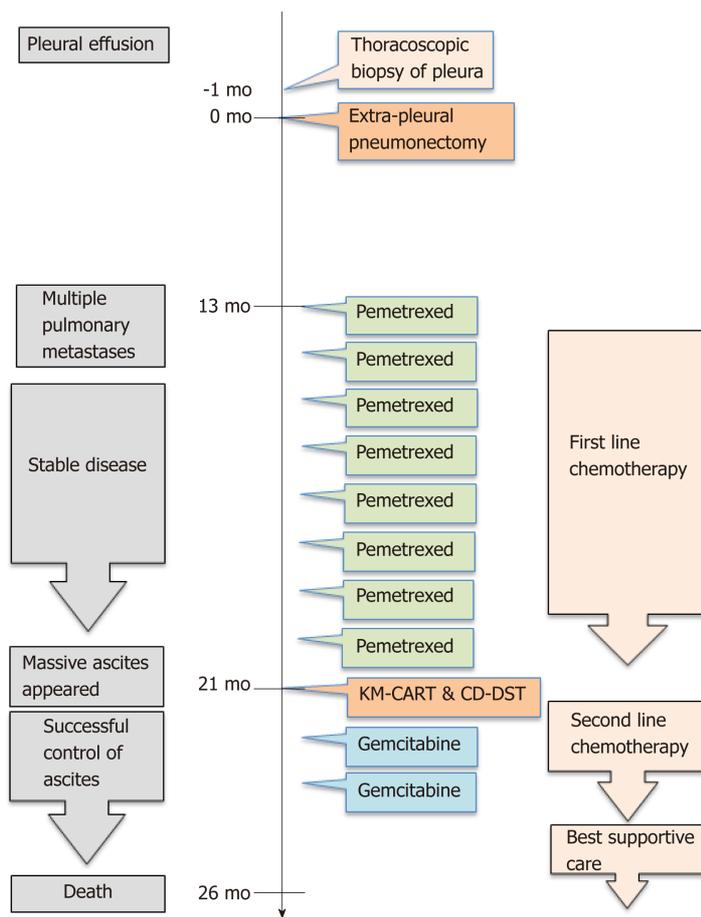


Figure 4 A flowchart depicting the timeline of treatment and outcomes. KM-CART: KM-cell-free and concentrated ascites reinfusion therapy; CD-DST: Collagen gel droplet-embedded culture drug sensitivity test.

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