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RESEARCH

Phase II study of Disulfiram and Cisplatin in Refractory Germ Cell Tumors. The GCT-SK-006 phase II trial

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Abstract

Background Multiple relapsed/refractory germ cell tumor (GCT) patients have extremely poor prognosis. Cisplatin resistant testicular GCTs overexpress aldehyde-dehydrogenase (ALDH) isoforms and inhibition of ALDH activity by disulfiram is associated with reconstitution of cisplatin sensitivity in vitro as well as in animal model. This study aimed to determine the efficacy and toxicity of ALDH inhibitor disulfiram in combination with cisplatin in patients with multiple relapsed/refractory GCTs.

Methods Disulfiram was administered at a dose of 400 mg daily until progression or unacceptable toxicity, cisplatin was administered at dose 50 mg/m^2 day 1 and 2, every 3 weeks. Twelve evaluable patients had to be enrolled into the first cohort, and if 0 of 12 patients had treatment response, the study was to be terminated. The results of the first stage of the trial are presented in this report.

Results Twelve patients with multiple relapsed/refractory GCTs were enrolled in the phase II study from May 2019 to September 2021. Median number of treatment cycles was 2 (range 1–6). None of patients achieved objective response to treatment, therefore the study was terminated in first stage. Median progression-free survival was 1.4 months, 95% CI (0.7–1.5 months), and median overall survival was 2.9 months 95% CI (1.5–4.7 months). Disease stabilization for at least 3 months was observed in 2 (16.7%) patients. Treatment was well tolerated, however, 5 (41.7%) of patients experienced grade 3/4 fatigue, 4 (33.3%) thrombocytopenia, 3 (25.0%) anemia, while 2 (16.7%) experienced neutropenia, nausea and infection.

Conclusions This study failed to achieve its primary endpoint and our data suggest limited efficacy of disulfiram in restoring sensitivity to cisplatin in multiple relapsed/refractory GCTs.

Keywords Germ cell tumours · Disulfiram · ALDH · Cisplatin · Refractory

Introduction

Germ-cell tumours (GCTs) are extraordinarily chemosensitive and resemble the clinical and biological characteristics of a model for the cure of cancer [1]. Nonetheless, a small proportion of patients do not have a durable complete remission with initial chemotherapy. Only 20–40% of them will be cured with the use of platinum-containing standard-dose or high-dose salvage chemotherapy with autologous stem cell transplantation (ASCT) [1–5]. Patients with multiple relapsed/refractory GCTs patients have extremely poor prognosis and long-term survival had been documented in < 5% [6–10].

Chemoresistance in solid tumors was associated with an upregulation of cancer stem cells (CSCs) markers [11, 12]. One of these markers is aldehyde dehydrogenase (ALDH) [11, 13]. This gene is expressed at high levels in stem cells and is involved in the regulation of stem cell function. Nine ALDH isoforms were identified potentially contributing to ALDH activity and they exhibit different expression patterns in different cancer types. However, mainly ALDH1 family members (ALDH1A1, ALDH1A2, and ALDH1A3)

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contribute to enhanced self-renewal, survival, and proliferation of CSCs [14]. Increased ALDH1 activity has been found in the stem cell populations of leukemia and some solid tumors [14]. Previously, we showed high *ALDH1A3* expression and increased ALDH activity were detected in refractory germ cell tumor lines. Moreover, we showed, that significantly higher ALDH1A3 expression was detected in testicular GCTs patients' tissue samples compared to normal testicular tissue [15, 16].

Disulfiram is a drug used to support the treatment of chronic alcoholism by producing an acute sensitivity to ethanol [17]. It works by inhibiting ALDH [18]. Numerous, in vitro and in vivo data showed activity of disulfiram in reversing cisplatin resistance in experimental models. In triple negative breast cancer, disulfiram treatment led to selective decrease in the ALDH-positive cell population while in ER-positive breast cancer cells it decreases ALDH1 activity [18–21]. Clinical experience with disulfiram in cancer patients remain limited. In non-small cell lung cancer, ALDH inhibition with disulfiram was associated with a reconstitution of cisplatin sensitivity in NSCLC cancer and some clinical responses, however, several trials are ongoing [22–26].

Previously, we showed that disulfiram in combination with cisplatin showed synergy for NTERA-2 and NCCIT cisplatin resistant GCTs cell lines. Moreover, disulfiram inhibits growth of NTERA-2 cisplatin resistant spheroids as well as xenograft growth in vivo in experimental model system [16]. Based on aforementioned data, we suggest that there is strong rationale to inhibit ALDH in testicular germ cell tumors (TGCTs). We supposed, that it may serve as an antitumor agent suitable for the drug repurposing in combination therapy in order to inhibit ALDH activity thus overcoming cisplatin resistance in refractory GCTs. We hypothesize that inactivation of ALDH by disulfiram recover cisplatin sensitivity in patients with progressing or relapsing germ cell cancer. This study aimed to determine the efficacy and toxicity of ALDH inhibitor disulfiram in combination with cisplatin in patients with multiple relapsed/refractory germ cell cancer.

Methods

Patients

This study included men 18 years or older, with ECOG performance status 0–2, histologically confirmed extracranial primary germ cell cancer, seminoma, or nonseminoma. Eligible patients included multiple relapsed/refractory GCTs e.g., at least 2 lines of previous chemotherapy and/or patients relapsing after high-dose chemotherapy or for patients non fit enough for high-dose chemotherapy. Primary mediastinal GCTs in first relapse were eligible too. Patient's disease must not be amenable to cure with either surgery or chemotherapy in the opinion of investigator. Patients must have adequate hematologic, liver, and renal functions. Patients with chronic alcoholism were excluded (For more details see www.clinicaltrials.gov, study identifier: NCT03950830).

The study protocol was reviewed and approved by the Ethical Committee of the National Cancer Institute in Bratislava, Slovakia. All the patients were required to provide written informed consent before enrollment.

Pre-treatment evaluation

All the patients were comprehensively evaluated with a complete medical history, physical examination, and laboratory and disease assessment. Brain imaging and bone scans were performed only in symptomatic patients.

Drug administration

- Cisplatin was administered intravenously 50 mg/m^2 day 1 and 2 every 3 weeks; disulfiram 400 mg once a daily, orally, continuously. No premedication or patient monitoring after administration of disulfiram was required. Patients took disulfiram after their evening meal. Courses was repeated every 21 days until progression or unacceptable toxicity. Treatment could continue at the discretion of investigator in case that patient benefit from the treatment. Standard emesis prophylaxis was used (e.g., dexamethasone, setron, aprepitant), before cisplatin. Anti-emetics were administered to subjects receiving disulfiram in chemo-free interval in case of nausea at the discretion of investigator.

Criteria to start and recycle chemotherapy

Each cycle was started if clinical status and biological data (granulocyte count > 1500/mm^3, platelets > 100,000 mm^3) and hemoglobin level > 9 g/dl allowed it. If chemotherapy couldn't be reinitiated due to toxicity, it was delayed until the limiting toxicity had resolved. Otherwise, patients were to receive full dose therapy. No dose modification of cisplatin is planned. Patients requiring a delay of > 2 weeks should go off protocol therapy.

Duration of therapy

A minimum of two cycles of the treatment were planned to be administered to each patient in the absence of unacceptable toxicity or disease progression. Patients might also discontinue protocol therapy in case of intercurrent illness which would in the judgment of the investigator affect patient safety, inability to deliver treatment or the request by patient.

Table 1 Patient characteristics

	N	%
Patients	12	100.0
Histology		
Seminoma	1	8.3
Nonseminoma	11	91.7
Primary tumor		
Gonadal	9	75.0
Retroperitoneal	1	8.3
Mediastinal	2	16.7
IGCCCG risk before 1st line of therapy		
Good prognosis	4	33.3
Intermediate prognosis	3	25.0
Poor prognosis	5	41.7
Number of previous lines of therapy		
3rd line	2	16.7
4th line	7	58.3
>4 lines	3	25.0
Sensitivity to cisplatin		
Sensitive	2	16.7
Resistant	4	33.3
Refractory	6	50.0
Sites of metastases		
Retroperitoneum	6	50.0
Other lymphadenopathy	7	58.3
Brain	2	16.7
Liver	6	50.0
Lung	9	75.0
Bone	3	25.0
Visceral non-pulmonary metastases	9	75.0
Number of metastatic sites		
1	1	8.3
2	5	41.7
≥3	6	50.0
ECOG		
0	4	33.3
1	6	50.0
2	2	16.7
Mean (range) of pretreatment markers		
AFP mIU/mL	398.4 (0.0–28706.6)	
βHCG IU/mL	2.4 (0.0–44397.3)	
LDH (μkat/L)	10.4 (1.9–20941.0)	

Evaluation of response and toxicity

A physical examination was performed and vital signs were assessed before each treatment cycle. Laboratory parameters, including serum tumor markers, were evaluated prior to every other cycle and one month after the end of treatment. Disease response assessment by CT scan was performed every 2 cycles (6 weeks).

The patients who received at least one dose of disulfiram and cisplatin were evaluated for their response according to standard RECIST (Response Evaluation Criteria in Solid Tumors) Criteria version 1.1 [27].

Primary endpoint of this study is overall response rate (ORR). The treatment was terminated in cases of disease progression, which was defined as significant marker progression (more than 50% increase) and/or radiological progression. Overall survival was measured from the day 1 of therapy. Toxicity was assessed after each cycle of therapy and scored using NCI-CTC Criteria (National Cancer Institute-Common Toxicity Criteria) version 4.1.

Role of sponsor

The sponsor of the study was the National Cancer Institute of Slovakia. The sponsor had no influence on the study design, treatment evaluation and/or statistical analysis of the study data.

Statistical considerations

Statistical and analytical plan

This is a phase II study to investigate the efficacy (as measured by ORR by RECISTs) of disulfiram and cisplatin in patients with multiple relapsed/refractory GCTs. The patients have to be not amenable for cure with either surgery or chemotherapy. A two-stage phase II design will be used for the patient accrual. Intention-to-treat analysis was used.

Study design, significance level and power

A Simon two-stage optimal design with type I error rate of 20% and power of 80% was utilized. The null hypothesis was an ORR of less than 5% and the alternate hypothesis was ORR equal or more that 15%. Consequently, 12 subjects were to be enrolled in the first stage. If no responses were determined in the initial stage, the study was to be concluded. If at least 1 patient achieved a partial or complete response, then 21 more subjects were to be accrued in the second stage for a total sample size of 33 subjects. If there were two or more subjects with partial or complete

response, the treatment regimen would be considered worthy of further investigation.

Statistical analysis

The study population was summarized using the mean or median (range) for continuous variables and the frequency (percentage) for categorical variables. The median follow-up period was calculated as the median observation time among all the patients. The progression-free survival (PFS) was calculated from the date of starting the treatment with disulfiram and cisplatin to the date of progression or death or to the date of the last follow-up. The overall survival (OS) was calculated from the date of starting the treatment with disulfiram and cisplatin to the date of death or last follow-up. The PFS and OS were estimated using the Kaplan–Meier product-limit method. Statistical analyses were performed using NCSS 10 (2015) software (Hintze J, 2015, Kaysville, Utah, USA).

Table 2 Study results

Variable	N	%
Objective response		
Complete remission	0	0.0
Partial remission	0	0.0
Disease stabilization	2	16.7
Progression	10	83.3
Type of response		
Favorable response	0	0.0
Unfavorable response	12	100.0

Results

Patient characteristics

Twelve patients with multiple relapsed/refractory GCTs were enrolled in the phase II study from May 2019 to September 2021. Median age was 36 years (range: 29–48 years). All patients were pretreated with at least 2 cisplatin-based therapies (median 4, range 2–7); 6 tumors (50.0%) were absolutely refractory to cisplatin and 9 patients (75.0%) had visceral non-pulmonary metastases (Table 1). Two patients (16.7%) were pretreated with high-dose chemotherapy with

Figure 1A: Kaplan-Meier estimate of progression-free survival (median PFS = 1.4 months 95% CI: 0.7–1.5 months)
Figure 1B: Kaplan-Meier estimate of overall survival (median OS = 2.9 months 95%CI: 1.5–4.7months)

Figure 1A

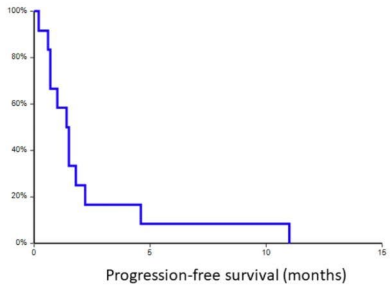
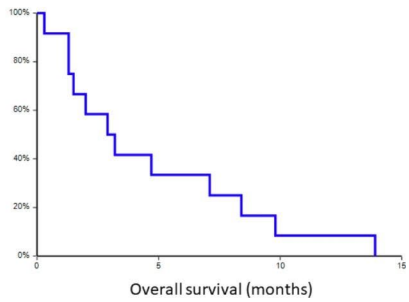


Figure 1B



autologous stem cell support. Six of 12 patients (50.0%) showed absolute platinum refractoriness, and 9 (75.0%) of patients had non-pulmonary visceral metastases. The median time from the diagnosis of metastatic disease to the start of study treatment was 18.9 months (range, 11.0–286.0 months).

Treatment outcome

None of patients achieved objective response to treatment, therefore the study was terminated in first stage. Disease stabilization for at least 3 months was observed in 2 (16.7%) patients (Table 2). Median number of administered treatment cycles was 2 (range: 1–6).

According to the statistical design, 12 patients were enrolled in the first cohort, and if fewer than 1 patient experienced ORR, the study was to be terminated. Given that none of the first 12 patients achieved partial or complete remission, the study was terminated in first stage.

During a median follow-up period of 3.1 months (range: 1.3–13.9 months), all (100%) patients experienced disease progression and died. Median progression-free survival was 1.4 months, 95% CI (0.7–1.5 months), and median overall survival was 2.9 months 95% CI (1.5–4.7 months) (Fig. 1).

Adverse events

Treatment was well tolerated, however, 5 (41.7%) of patients experienced grade 3/4 fatigue, 4 (33.3%) thrombocytopenia, 3 (25.0%) anemia, while 2 (16.7%) experienced neutropenia, nausea and infection. Other grade 3/4 adverse events included syncope, tumor related pain, constipation, dyspnea, mineral disbalances and sensory polyneuropathy (Table 3). At least 1 grade 3/4 adverse event experienced 10 (83.3%) of patients.

Table 3 Main Grade 3 or 4 Adverse Events per Patient According to NCI-CTC (version 4.03) Classification (N = 12)

Variable	N	%
Fatigue	5	41.7
Thrombocytopenia	4	33.3
Anemia	3	25.0
Neutropenia	2	16.7
Nausea	2	16.7
Infection	2	16.7
Syncope	1	8.3
Tumor related pain	1	8.3
Constipation	1	8.3
Dyspnea	1	8.3
Hyponatremia	1	8.3
Hyperkalemia	1	8.3
Polyneuropathy sensory	1	8.3

Discussion

In this phase II study disulfiram was not able to reverse cisplatin sensitivity in patients with multiple relapsed/refractory GCTs. Outcome of the patients was consistent with previous data in refractory GCTs. Moreover, we were not able to identify even a subgroup of patients that could potentially benefit from the treatment. In vitro data suggest efficacy of disulfiram and cisplatin in embryonic GCTs cells lines [16]. In our trial, one patient had pure embryonal carcinoma (EC) and 5 patients had EC component within mixed GCTs, however, nor response was observed in any of these patients.

Outcome of multiple relapsed/refractory GCTs remains extremely poor [6]. Numerous strategies are utilized to overcome cisplatin resistance in GCTs, however, current results including this trial remains unsatisfactory [6, 8–10, 28–30]. A meta-analysis of several phase II trials that analyzed the effectivity of targeted agents used in monotherapy in refractory GCTs revealed median PFS and OS were only 1.0 month and 4.7 months, respectively, observation consistent with the results of the present trial [7].

Despite promising in vitro data, disulfiram failed to reverse cisplatin resistance in clinical setting. One of the explanations could be insufficient dose of disulfiram. In our trial we used 400 mg daily, based on suggested dose for treatment of chronic alcoholism [17]. In phase I and II trials in cancer patients, evaluated dose of disulfiram vary from 40 mg to 2000 mg daily [22–26]. Therefore, we cannot exclude that higher dose of disulfiram could be more efficacious. Moreover, in our trial we didn´t evaluated percentage of inhibition of ALDH in peripheral lymphocytes. Another explanation of study failure could be absence treatment target. Measurement of ALDH expression in tumor tissue could probably answer this question, however, according to our prior evaluation in 216 patients, more that 70% of GCTs express ALDH, with the highest frequency of the ALDH1A3 expression was found in teratomas (77.8%), with decreasing trend in germ cell carcinoma in situ (GCNIS) (74.6%), embryonal carcinomas (71.0%), in choriocarcinomas (63.6%), yolk sac tumors (46.7%) and, at least, in seminomas (42.0%) [16]. Therefore, we don´t suppose, that absence of treatment target could be responsible for patient's outcome. Copper is mineral, that act synergistically with disulfiram in ALDH inhibition [21, 31]. Currently several trials aimed to evaluate disulfiram in cancer treatment utilized copper as adjunctive therapy. Therefore, we can´t exclude that copper could increase the efficacy of evaluated therapy. Moreover, another possibility is that mechanism of cisplatin resistance in GCTs is more complex and single inhibition of ALDH is not sufficient in clinical setting to overcome this resistance. Clinical experience

with disulfiram in cancer patients remains limited, however, available data suggest that its efficacy at the most modest [22, 24, 25]. Recently it was observed that gain of 3p25.3 could be responsible for cisplatin resistance in proportion of GCTs patients [32], however, other mechanism remains to be elucidated.

In conclusion, this study failed to achieve its primary end point and our data suggest limited efficacy of disulfiram in restoring sensitivity to cisplatin in multiple relapsed/refractory germ cell tumors. New treatment strategies are vigorously awaited to overcome cisplatin resistance of refractory GCTs. We suggest, that evaluation of new treatments should include broad spectrum of preclinical in vitro and in vivo models, including cell line with different mechanism of cisplatin resistance.

Abbreviations

AFP	alpha-fetoprotein
ECOG	Eastern cooperative oncology group
HCG	human chorionic gonadotropin
IGCCCG	International Germ Cell Cancer Collaborative Group
LDH	lactate dehydrogenase
LN	lymph nodes

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Author contributions M-M J-M and M.CH participated in the conception and design of this study. D-S and M-R participated in data validation, M-Ma, V-DA, K-K, P-L, J-O, Z-O, P-P, K-R, Z-SM, and M-CH acquired, analyzed and interpreted the data. M-M drafted the article, and all the authors reviewed it critically for its important intellectual content.

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Declarations

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval All the procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent Informed consent was obtained from all individual participants included in the study.

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