Published in final edited form as:

Invest New Drugs. 2011 April ; 29(2): 340–346. doi:10.1007/s10637-009-9383-9.

A phase I dose-escalation, safety and pharmacokinetic study of the 2-methoxyestradiol analog ENMD-1198 administered orally to patients with advanced cancer

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Summary

Background—2-methoxyestradiol (2ME2) is an estradiol-17 β metabolite with antiproliferative and antiangiogenic activities. ENMD-1198 is an analog of 2ME2 which was developed to decrease the metabolism and increase both the bioavailability and antitumor activities of the parent molecule. This first-in-human phase I study evaluated the tolerability, pharmacokinetics and preliminary evidence of activity of ENMD-1198 in advanced cancer patients.

Methods—Eligible patients received ENMD-1198 orally once daily in Part A (standard 3+3 dose escalation design), or in Part B (accelerated dose escalation design). Cycle 1 consisted of 28 days daily dosing followed by a 14-(Part A) or 7-(Part B) day observation period, then continuously in 28 day cycles thereafter.

Results—A total of 29 patients were enrolled in 12 dose cohorts (5 to 550 mg/m²/d). The most common drug-related toxicities were Grade 1/2 fatigue (55%), nausea and vomiting (37%), and constipation (34%). Two DLTs (Grade 4 neutropenia) occurred at 550 mg/m²/day, and 425 mg/m²/d was declared the maximum tolerated dose. ENMD-1198 was absorbed rapidly with a T_{max} of 1–2 h. Exposure to ENMD-1198 (C_{max} and AUC_{0–24hr}) increased linearly with dose. The mean terminal half-life was 15 h. A 3-fold accumulation was found after multiple doses. Five patients achieved stabilization of disease for at least 2 cycles, three of whom (with neuroendocrine carcinoma of pancreas, prostate cancer and ovarian cancer) demonstrated prolonged stabilization ranging from 8–24.5 cycles.

Conclusion—ENMD-1198 is well-tolerated with a pharmacokinetic exposure profile compatible with once daily dosing. The recommended phase II dose of ENMD-1198 is 425 mg/m²/d. Early evidence of prolonged disease stabilization in pre-treated patients suggests ENMD-1198 is worthy of additional investigation.

Keywords

ENMD-1198; Phase I; Pharmacokinetics

Introduction

ENMD-1198 (2-methoxyestra-1,3,5(10)16-tetraene-3-carboxamide), also known as C24, 883, or ENMD-0998, is an analog of 2-methoxyestradiol (2ME2, Panzem®; EntreMed, Inc.). 2ME2 is an endogenous, estradiol-17 β metabolite existing at low levels in human plasma which has antiproliferative and antiangiogenic activities at pharmacologic doses [1–4]. Previous phase I studies with a 2ME2 delivered as a capsule formulation showed the compound was well tolerated with evidence of anti-cancer activity in patients with advanced cancer, but the systemic exposure was below the expected therapeutic range because of low oral bioavailability and metabolism [5]. A Nano-Crystal Dispersion formulation of 2ME2 (2ME2 NCD) was developed by milling the drug substance particle size into submicron size particles and formulating as nanoparticle drug crystals, which resulted in increased oral bioavailability and enhanced anticancer activity in animal models [6–8]. 2ME2 NCD was demonstrated to be well tolerated in cancer patients and clinical benefit was seen in some patients, but the anti-tumor activity was modest in heavily pretreated patients [9]. To generate metabolically stable analogues with improved antitumor activities, a series of analogues of 2ME2 were developed. ENMD-1198 was found to be the most promising analogue in preclinical studies.

ENMD-1198 exhibited significant antiproliferative and antiangiogenic activities both in vitro and in vivo in preclinical cancer models [10, 11]. The antiproliferative mechanism of ENMD-1198 is thought to reflect its ability to inhibit microtubule polymerization by binding to β -tubulin at or near the colchicine binding site leading to cell cycle arrest and induction of apoptosis. Similar to the antiangiogenic activities of 2ME2 [12, 13], ENMD-1198 can also decrease production of vascular endothelial growth factor (VEGF) and inhibit the expression of hypoxia-inducible factor (HIF)-1 α [14]. Potent antitumor activity was demonstrated in tumor-bearing mice at doses ranging from 50–400 mg/kg/day. In pre-IND toxicity studies, the 28-day no-observed-adverse-effect-level (NOAEL) and lowest-observed-adverse-effect-level (LOAEL) were significantly different between rats (5 mg/kg/day or 30 mg/m², and 10 mg/kg/day or 60 mg/m², respectively) and dogs (20 mg/kg/day or 400 mg/m², and 30 mg/kg/day or 600 mg/m², respectively). Common toxicities across species at high doses were found in organs with high mitotic activity, such as bone marrow, lymphoid organs, the gastrointestinal (GI) tract, and testes. Most toxicities resolved after a 14 day drug free period [15].

The goals of this first-in-man dose-escalation phase I study were: 1) to assess the safety and tolerability of escalating doses of ENMD-1198 in patients with advanced cancer; 2) to determine the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of ENMD-1198 administered orally once daily; 3) to determine the plasma pharmacokinetics of ENMD-1198 in cancer patients; 4) to explore any early evidence of benefit in cancer patients by evaluating objective tumor response and/or decreases in relevant tumor markers on therapy.

Patients and methods

Patient selection

Patients aged 18 years or older with advanced cancer for which no effective therapy existed, who met the modified RECIST for measurable lesion(s) or who had disease that could be followed with a measurable serologic tumor marker, with an anticipated life expectancy of at least three months and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 were eligible for enrollment. Adequate organ function included AST and ALT <2.5 times upper limit of normal (ULN), alkaline phosphatase <2.5×ULN, direct bilirubin <1.5×ULN, creatinine <1.5×ULN, absolute neutrophil count >1500 cells/mm³, platelets >100,000/mm³ and hemoglobin >9.0 g/dL. Mandated washouts from previous treatments were 2 weeks for chemotherapy or radiotherapy and 30 days for prior systemic treatment within a clinical trial. Because of theoretical concerns relating to possible neurotoxicity from an agent with anti-tubulin activity, patients with baseline sensory neuropathy of Grade 2 or greater were excluded from the study. The study was conducted in accordance with the Declaration of Helsinki and approved by an independent review board. Written informed consent was obtained from each patient.

Study design and drug administration

This was a two-part single-center phase I dose-escalation trial. Part A involved a classical 3+3 dose escalation cohort design starting at 1/12th of the allometrically scaled LOAEL as determined in the most sensitive species (rat). Five cohorts (dosing levels) were initially planned (5, 10, 20, 30 and 40 mg/m²/day). Three patients were enrolled into each cohort initially. All toxicities were assessed according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAE v3). Dose limiting toxicity (DLT) was defined as the occurrence of any treatment-related Grade 3 or greater nonhematologic adverse events (AEs) or Grade 4 or greater hematologic AEs during the first cycle of administration of ENMD-1198 (nausea, vomiting and diarrhea, unless adequate supportive care was given, and alopecia were excluded from the determination of DLT). If one patient of any cohort developed a DLT the cohort was expanded to up to six patients. If two or more patients in any cohort developed DLTs, patient accrual to the cohort and dose escalation were stopped and the next lowest dose would be considered the maximum tolerated dose (MTD). Due to the absence of significant drug-related toxicity in Part A, the protocol was amended to continue dose escalation within Part B. In Part B more aggressive dose escalation and single patient cohorts were employed until drug-related toxicities of at least grade 2 in nature developed. Nine cohorts were planned (60, 120, 180, 240, 320, 425, 550, 725, and 1000 mg/m²/day). The occurrence of Grade 2 drug-related toxicities in Cycle 1 triggered expansion of the cohort and all subsequent cohorts along standard 3+3 criteria as outlined above. Dose escalation steps at this point were also reduced to increases of 33%. All enrolled patients who received at least one dose of ENMD-1198 constituted the safety population for demographic characteristics analyses and safety analyses.

ENMD-1198 was supplied as a NanoCrystal® Dispersion (NCD) as 0.4%, 3%, and 5% suspensions in HDPE bottles and was provided by Elan Drug Delivery, Inc. The investigator calculated the volume of dose to be administered to each patient based on body weight and

height obtained at the screening visit. Dosages were not recalculated in the absence of significant toxicities (see below). After enrollment, patients in each cohort received once daily oral doses of ENMD-1198 in 28-day Cycle 1. Patients were required to fast overnight prior to dosing on Day 1 and Day 28, and were required to refrain from eating for 1 h before and 1 h following all doses. After finishing Cycle 1, patients in Part A underwent a 14-day observation period while those in Part B underwent a 7-day observation period. Each subsequent treatment cycle in both parts was for 28-days without additional observation periods. No inpatient dose escalation was allowed. If the patient developed Grade 4 hematological or Grade 3 non-hematological treatment-related toxicity, the study drug was stopped until recovery to grade <2. Stable or responding patients who recovered from these severe toxicities within 14 days could restart the study drug at a lower dose level while those who did not recover within 14 days were removed from study. Only one de-escalation was allowed per patient.

Pretreatment and follow-up studies

Medical history, physical examination, routine blood and urine tests, 12-lead ECG, concurrent medications, directed neurological examination, measurable and non-measurable tumor lesions or tumor markers were assessed during the screening visit. Routine safety blood tests, AEs and concurrent medications were monitored weekly throughout the study. ECG's were repeated on day 29 of cycle 1 and after the discontinuation of study. Directed neurological examination was repeated monthly for cycles 2–4 and then every other cycle until the patient came off the study. Clinical benefit was assessed radiologically per RECIST every two cycles in those with measurable disease, and/or by evaluation of serologic tumor markers on day 1 of each cycle.

Pharmacokinetic sampling and analysis

For pharmacokinetic assessment, blood samples were collected immediately prior to the first dose on day 1 of cycle 1 for each dose level and then at 0.5, 1, 2, 4, 6, 8, and 24 h (+/- 5 min) following dosing. In addition, for cycle 1 only, additional blood samples were obtained at 24, 48 and 72 h (+/- 30 min) after the last dose of ENMD-1198 on day 28, and on days 29, 30 and 31 of the observation period. For subsequent cycles, only one blood sample was collected immediately prior to the day 1 dose administration for each cycle.

A validated LC/MS/MS assay was used to determine concentrations of ENMD-1198 by Colorado State University (Fort Collins, CO). The pharmacokinetic parameters were determined via noncompartmental analyses using PK Solutions 2.0 (Summit Research Services, Montrose, CO). ANOVA or comparable nonparametric statistical methods were used to make dose group comparisons. Pharmacokinetic analyses included plasma area under the plasma concentration-time curve (AUC), elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), minimum plasma concentration (C_{min}), time when C_{max} occurs (t_{max}), estimated steady-state plasma concentration (C_{pss}), total clearance (CL), volume of distribution (V_z), and accumulation.

Results

Between January 2006 and January 2009, a total of 29 patients were enrolled in 12 dose cohorts (5 to 550 mg/m²/d). Their demographic features are summarized in Table 1.

All enrolled patients received at least one dose of ENMD-1198. A total of 85.5 cycles of therapy were completed (mean per patient = 2.9; range: 0.5–24.5). Dose cohorts, number of patients in each cohort and completed cycles are listed in Table 2.

Toxicities

No drug-related Grade 3/4 toxicities occurred across the 5–425 mg/m²/d dose range (Table 3). However, in dose cohort 12 (550 mg/m²/day) two patients experienced DLTs (grade 4 neutropenia). Both patients had ENMD-1198 held for two weeks and restarted it at 425 mg/m²/day after their toxicities recovered to grade 1 or less. They tolerated the lower dose well and had no further grade 3/4 toxicities. After cycle 2, both of them were removed from the study for progressive disease. There were no other drug-related dose interruptions or reductions in other cohorts. No life-threatening AEs or treatment-related deaths occurred in the study. Four more patients were enrolled into the 425 mg/m²/day cohort, with a total of 2 needing replacement (one for early clinical progression and one for consent withdrawal, both in the absence of significant drug-related toxicities). In total, 3 patients completed cycle 1 at 425 mg/m²/day with little evidence of toxicity and this dose level was declared the MTD.

The numbers of patients who had Grade 1/2 drug-related adverse events (all cycles) in each cohort are listed in Table 3, with little suggestion of a dose effect until dose cohort 12 was reached. In cohort 12, in addition to the 2 DLTs, there were also multiple examples of grade 1 or 2 toxicities (2 cases of fatigue, 2 of alopecia, and 1 each of anorexia, anemia, thrombocytopenia, diarrhea, liver function tests abnormality, gastroesophageal reflux disease, neuropathy and constipation). Patients who had the same adverse event in subsequent cycles counted once. Across all dose cohorts, the most common drug-related toxicities (all grades) were fatigue (55%), nausea and vomiting (37%), and constipation (34%).

Pharmacokinetics

ENMD-1198 was absorbed rapidly with the T_{max} typically occurring within the first two hours after oral administration (Table 4 and Fig. 1). The longer average T_{max} after 425 mg/m² was due to delayed ENMD-1198 absorption in one patient.

Plasma exposures increased proportionally with dose from 5 to 550 mg/m². No evidence of saturation of absorption was found over the dose range in this study. Terminal elimination half-life after a single dose was variable independent of the administered doses (Table 4). The mean terminal half-life was 15 h.

After multiple doses, accumulation was determined by comparison of plasma concentration at 24 h after dosing on Day 28 to that of Day 1 ($C_{trough, Day 28} / C_{trough, Day 1}$). The ratio was 2.93 ± 0.64 (range of 1.61 to 4.04) over the dose range of 5 to 425 mg/m² (data not shown).

Due to dose interruptions relating to the DLTs at 550 mg/m² it was not possible to accurately assess accumulation for this dose over the same time frame.

Antitumor activity

There were no objective tumor responses. However, stable disease (SD) for at least 2 cycles, sometimes in association with minor responses, was seen in five patients. One of them (a 55 year old, female, Caucasian, with neuroendocrine carcinoma of pancreas previously treated only with radiotherapy to her bones without any chemotherapy) experienced a 25% shrinkage of her measurable lesions at 60 mg/m² for 24.5 cycles (22 months and 3 weeks) and was finally removed from the study for clinical progression. One patient with prostate cancer experienced stable PSA at 30 mg/m² for 10 cycles (9.5 months) and one patient with ovarian cancer experienced SD at 550 mg/m² for 8 cycles (7 months and 3 weeks). Another two patients (with prostate cancer and mesothelioma) experienced SD at 240 mg/m² and 425 mg/m², respectively for 2 cycles but progressed after 3 cycles.

Discussion

ENMD-1198 is an analog of 2ME2 designed to decrease the metabolism of 2ME2 and to increase its bioavailability and antitumor activities. Non-clinical data showed that ENMD-1198 exhibited significant anticancer effects and good tolerance when administered at predicted efficacious doses. However, there were significant inter-species differences in the NOAEL and LOAEL determined in non-clinical studies, with rats being significantly more sensitive to the drug than dogs, which resulted in the low starting dose used in this study. This phase I study evaluated the safety, tolerability, pharmacokinetic exposures and any preliminary evidence of anti-cancer activity of ENMD-1198 in cancer patients for the first time.

In this study, ENMD-1198 was well-tolerated across a range of doses from 5 to 425 mg/m²/day. An accelerated dose escalation was introduced after it became clear that humans are not as sensitive as rats were to ENMD-1198. Grade 1/2 fatigue and gastrointestinal events including nausea, vomiting, and constipation were the most common drug-related toxicities, which were consistent with the results in preclinical studies. Although fatigue was the main DLT in the phase I study of 2ME2 NCD [16], no severe fatigue was found in this study. No DLTs occurred until the 550 mg/m² cohort was reached, when two of three patients experienced reversible grade 4 neutropenia in Cycle 1 in conjunction with an increase in additional grade 1/2 toxicities (Table 3). Of note, no severe myelosuppression has been found in clinical trials of 2ME2 NCD [9, 16]. At 425 mg/m² cohort, grade 1/2 toxicities included three cases of nausea/vomiting, two of fatigue, and one each of anorexia, anemia, liver function tests abnormality and constipation (Table 3). However, because of the high background frequency of grade 1 and 2 toxicities in any advanced cancer patient population it is difficult to be certain whether there was evidence of a true dose-toxicity relationship below 550 mmg/m².

The pharmacokinetic analyses showed that the ENMD-1198 NanoCrystal® Dispersion formulation resulted in ENMD-1198 being absorbed rapidly with a T_{max} of 1–2 h. The relationship between pharmacokinetic profile (dose normalized C_{max} and AUC_{0–24hr}) and

dose was linear without evidence of saturation of absorption over the dose range used in the current study. A 3-fold accumulation was found after multiple doses, which was similar to the accumulation of 2ME2 NCD after multiple doses [16]. ENMD-1198 had a mean terminal half-life time of 15 h, longer than that of both 2ME2 capsule formulation and 2ME2 NCD [5, 16], and compatible with a once daily dosing schedule for ENMD-1198. These data indicate that the PK objective of the 2ME2 chemical analog program which led to the discovery of ENMD-1198 was met.

While there was no evidence of ENMD-1198 clearance saturation at 550 mg/m², exposures were significantly higher compared to the next lowest dose level. These differences alone may well be responsible for the major difference in the tolerability between 550 and 425 mg/m² dosing. However, in preclinical studies the differences in tolerability between rats and dogs did not appear to relate to differences in exposure to the parent compound across species (unpublished preclinical data on file at Entremed; Fig. 2). One hypothesis is that a metabolite responsible for much of the toxicity is differentially produced between species; however, radio-labeling studies have yet to identify such a metabolite (unpublished preclinical data on file at Entremed). Since the MTD in humans appears more similar to that of dogs than rats (Fig. 2), the assumption is that human metabolism of ENMD-1198, with respect to the production of this putative toxic metabolite, more closely resembles that of dogs than rats. In the absence of knowing what this metabolite is, whether significant differences in its production and/or clearance in humans also contributed to the differences in tolerability between 425 and 550 mg/m², cannot be excluded.

Out of the 29 patients enrolled in this study, although no objective responses were seen, 5 patients appeared to derive clinical benefit from ENMD-1198 treatment. Three patients demonstrated marked stabilization of their diseases lasting for 24.5 cycles, 10 cycles and 8 cycles. The efficacy of ENMD-1198 was seen in different tumor types (neuroendocrine carcinoma of pancreas, prostate cancer and ovarian cancer) and over a broad range of doses (30, 60 and 550 mg/m²).

Importantly, although the major toxicity of ENMD-1198 at 550 mg/m² was neutropenia, which could be mechanistically related to efficacy as well as toxicity, no severe myelosuppression has been noted in clinical trials of the active progenitor compound 2ME [9, 16]. In the current study, evidence of clinical activity, including minor responses and prolonged disease stabilization, was similarly noted at dose levels well below those producing myelosuppression. Together these data suggest that the anticancer efficacy of ENMD-1198 may occur independent of its effects on the bone marrow.

In conclusion, ENMD-1198 is extremely well tolerated in humans at doses up to 425 mg/m², with dose-proportional increases in pharmacokinetic exposures over the 5–550 mg/m² range. Unlike its progenitor compound 2ME2, pharmacokinetic exposures to ENMD-1198 are fully compatible with once daily dosing. Neutropenia occurs as a dose limiting toxicity at 550 mg/m²/day, the next highest dose level assessed in this study. The basis for the sharp distinction in toxicity between 425 and 550 mg/m²/day may be explained by the differences in exposure to the parent compound, but the possibility of significant differences in exposure to an unidentified toxic metabolite between these two dose levels also has to be considered.

Similar significant differences in the tolerability of the parent compound across species despite comparable exposures to the parent compound are also consistent with this hypothesis. Preliminary evidence of clinical benefit was seen at doses from 60–550 mg/m², in terms of minor shrinkage and prolonged disease stabilization in patients with neuroendocrine carcinoma of the pancreas, prostate and ovarian cancer. Although the dose-limiting toxicity of neutropenia could be addressed through the use of myeloid growth factor support, the absence of myelosuppression with 2ME2 and the suggestion of clinical activity of ENMD-1198 at dose levels below the DLT level suggest that there may not be a close relationship between myelosuppression and clinical activity for this compound. Consequently, the well-tolerated MTD of 425 mg/m²/d will be the RP2D explored in subsequent studies.

Acknowledgments

Mark Morrow (Clinical Study Co-ordination), University of Colorado Cancer Center.

Abbreviations

2ME2	2-methoxyestradiol
NCD	NanoCrystal® Dispersion
VEGF	Vascular endothelial growth factor
PK	Pharmacokinetic
DLT	Dose limiting toxicity
MTD	Maximum tolerated dose
LC/MS/MS	Liquid chromatography-tandem mass spectrometry
ECOG	Eastern Cooperative Oncology Group
CTCAE v3	Common Terminology Criteria for Adverse Events, Version 3.0
RECIST	Response Evaluation Criteria In Solid Tumors

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Fig. 1.
Mean plasma concentration-time profiles of ENMD-1198 in cancer patients after a single oral dose

Fig. 2.

Cross-species comparison of ENMD-1198 PK (C_{max}) by dose, DLT and MTD. The mean C_{max} on day 1 and MTD from 28-day toxicokinetic studies in rats and dogs (15) were plotted as a function of dose following conversion from mg/kg to body surface area dosing. These values are compared to mean values from the clinical dose escalation (Fig. 1)

Table 1Patient demographics (*N*=29)

	Number
Gender	
Male	14
Female	15
Median age (range)	60 (35–77)
ECOG performance status	
0	7
1	22
Race	
White	28
Asian	1
Prior anticancer therapies	
Chemotherapy	26
Radiotherapy	19
Hormonal	9
Immunotherapy	4
Investigational agent	5
Median prior drug regimens (range)	3 (0–8)
Tumor types	
Prostate	7
Ovarian	5
Colorectal	5
Melanoma	2
Breast	2
Others (Kidney, NSCLC, neuroendocrine carcinoma of pancreas, Adenocystic carcinoma, Pancreas, Bladder, Mesothelioma, Adrenal)	1 each

ECOG Eastern Cooperative Oncology Group, *NSCLC* non-small cell lung cancer

Table 2

Dose cohorts, number of patients and completed cycles

Dose cohort	ENMD-1198 (mg/m ² /d)	Number	completed cycles (mean)
1	5	4	1.5
2	10	3	1.7
3	20	3	1.7
4	30	3	4.3
5	40	3	1.3
6	60	1	24.5
7	120	1	2
8	180	1	2
9	240	1	2
10	320	1	1
11	425	5	1.6
12	550	3	3.7

Table 3

Grade 1/2 study drug-related adverse events by cohort (all cycles). No study-drug related toxicities greater than grade 2 occurred below 550 mg/m²/day^a

Dose cohort	1	2	3	4	5	6	7	8	9	10	11	12
Fatigue	3	1	2	2	2	-	-	1	1	-	2	2
Anorexia	2	-	-	-	-	-	-	1	-	-	1	1
Anemia	2	1	1	1	1	-	-	-	-	-	1	1
Neutropenia	-	1	-	-	-	-	-	-	-	-	-	-
Thrombocytopenia	-	-	-	-	-	-	-	-	-	-	-	1
Nausea/Vomiting	2	1	-	1	1	-	-	1	-	-	3	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	1
Alopecia	1	-	1	-	-	-	-	-	-	-	-	2
LFTs	-	-	-	-	-	-	-	-	-	-	1	1
Facial flushing	1	-	-	-	-	-	-	-	-	-	-	-
GERD	2	1	1	1	-	-	-	-	-	-	-	1
Change in taste	1	-	-	-	-	-	-	-	-	-	-	-
Neuropathy	-	3	1	-	-	-	-	1	-	1	-	1
Hypotension	-	-	-	-	1	-	-	-	-	-	-	-
Constipation	3	-	3	1	2	-	-	-	1	1	1	1

LFTs liver function tests; GERD Gastroesophageal reflux disease

^a At 550 mg/m²/day 2 cases of grade 4 neutropenia within Cycle 1 (DLTs) occurred

Table 4

Non-compartmental pharmacokinetic parameters of ENMD-1198 in cancer patients after a single oral dose

Dose cohort (mg/m ² /day)	n	C _{max} (ng/mL)	T _{max} (hours)	AUC _{0-24hr} (ng·hr/mL)	Half-life (hours)
5	4	68.75±48.50	1.25±0.50	312.35±175.38	12.16±4.22
10	3	141.20±28.47	1.67±0.58	1073.83±156.28	10.57±4.42
20	3	418.66±72.53	1.33±0.58	2138.07±384.60	11.03±1.85
30	3	677.80±125.78	1.00±0.00	3914.43±1336.73	19.45±7.66
40	3	675.83±280.76	1.33±0.58	3616.23±1880.32	8.98±4.22
60	1	1032.20	1.00	4901.00	11.82
120	1	1823.10	1.00	10387.00	10.66
180	1	2904.10	1.00	14789.00	29.80
240	1	2559.90	1.00	16277.00	16.15
320	1	3851.00	2.00	42184.00	9.70
425	5	2420.94±1098.82	6.20±9.96	24816.06±14612.08	18.40±13.04
550	3	4319.67±2006.78	1.67±0.58	45223.33±18198.49	17.93±4.11