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Original Article

The role of low-dose decitabine combined with chemotherapy in the treatment of recurrent and refractory gastric cancer: a single-center, single-arm clinical study

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Abstract: Low-dose decitabine is a safe drug which can inhibit DNMT activity to promote tumor cell apoptosis without inducing cell death. This study investigated the clinical efficacy of low-dose decitabine combined with chemotherapy on the treatment of recurrent and refractory gastric cancer. A total of 227 patients with refractory gastric cancer were retrospective analyzed, including 127 patients treated by low-dose decitabine combined with chemotherapy drugs (observation group) and 100 cases treated by chemotherapy alone (control group). $CD4^+$, $CD8^+$, and $CD4^+/CD8^+$ proportion in peripheral blood lymphocytes were measured by ELISA. Adverse reactions were evaluated by the common toxicity classification criteria according to WHO. The disease control rate (83%) and median survival time (10 months) in observation group were significantly higher than those in control group ($P < 0.05$). $CD3^+$ showed no obvious changes between before and after treatment ($P > 0.05$). However, $CD4^+$ and $CD4^+/CD8^+$ proportion in both groups were significantly increased after treatment ($P < 0.05$). Compared with control, $CD4^+$ and $CD4^+/CD8^+$ proportion were significantly increased in observation group after treatment ($P < 0.05$). The incidence of adverse reactions in observation group was markedly lower than that in control group ($P < 0.05$). The partial remission rates were 47.2% and 43.0%, whereas the disease control rates were 87.4% and 81.0% in observation group and control group, respectively. The application of low-dose decitabine combined with chemotherapy can improve the clinical efficacy, enhance immune function, while reduce the incidence of adverse reactions in patients with refractory gastric cancer.

Keywords: Low dose, decitabine, combined chemotherapy, tegafur, refractory gastric cancer

Introduction

Gastric cancer is one of most common malignant tumors around the world with a high incidence and mortality in China [1]. The best treatment approach for gastric cancer is surgical resection. However, because of its occult onset and changeable symptoms, more than 50% of patients have progressed at the time of being diagnosed and missed the best period of radical surgery. There are 50-60% of patients with recurrence in situ or metastasis within two years after radical gastrectomy even in early stage [2]. For patients with refractory gastric cancer, systemic chemotherapy-based comprehensive treatment is an important method, and clinical data show that the combined chemotherapy is better than monotherapy [3]. Traditional treatments, including chemotherapy, radiotherapy, and surgery, have achieved efficacy in a variety of solid tumors and hematologic malignancies. However, most patients ultimately develop resistance to these therapies and more than 90% of cancer patients died of recurrent and metastatic disease. Taking into account the defects of traditional therapies in the treatment of refractory solid tumors relapse, new therapy is urgently required to improve the status [4]. Decitabine has been used in clinic for more than 10 years with high safety. It is a cytosine mimics and DNA methyltransferase (DNMT) inhibitor that can silent gene expression by inhibiting DNA methylation through incorporating into DNA double-strand [5]. De-

citabine has showed good therapeutic effects on MDS, CML, and other hematological malignancies. However, its prospect in the treatment of solid tumors has not been fully explored [6]. Low dose of decitabine can inhibit DNMT activity without inducing cell death. Decitabine can activate the expression of tumor suppressor genes, cell cycle regulatory genes, and other silencing genes to promote tumor cell apoptosis or inhibit cell proliferation [7]. In this study, we initially conducted one-arm clinical trial of metastatic and refractory pancreatic cancer/colorectal cancer/gastric cancer to evaluate the efficacy and safety of decitabine. Meanwhile, it may provide basis to establish standard epigenetic treatment program.

Patients and methods

Clinical information

A total of 227 gastric cancer patients (pathologically confirmed and inoperable squamous cell carcinoma and adenocarcinoma) in our hospital from April 2015 to October 2017 were enrolled, including 127 patients treated with low-dose decitabine combined with chemotherapy as observation group and 100 patients treated with simple chemotherapy as control group. All patients were found lesions, and heart, liver and kidney functions were normal before chemotherapy. There were no significant differences between the two groups in terms of age, sex and pathological classification. Inclusion criteria: ① meet the diagnostic criteria of squamous cell carcinoma and adenocarcinoma that cannot afford surgery and was suitable for chemotherapy; ② age 25 - 60 years; ③ no chemotherapy contraindications; ④ sign the informed consent. Exclusion criteria: ① extensive metastases of gastric cancer; ② severe liver, kidney, heart diseases or coagulation disorders; ③ poor compliance; ④ existence of mental illness. This study was approved by the ethics committee and all the subjects had signed informed consent.

Treatment method

Control: The subjects received tegafur capsule orally after the meal at 80 mg/m^2 for twice a day.

Observation group

The subjects received intravenous infusion of decitabine at 10 mg/d on the 1st-5th day and tegafur capsule orally after the meal at 60 mg/m^2 for twice a day on the 6th-7th day.

The two therapies were repeated every three weeks. Electrocardiography and hepatorenal function were monitored to prevent potential adverse reaction.

Observation index

All patients underwent at least 2 cycles of chemotherapy.

Curative effect index

According to the RECIST1.1 standard, the treatment effect was divided into complete remission, partial remission, stability, and progress. The total effective rate = (complete remission + partial remission)/total population, disease control rate = (total population - progress)/total population. The survival time of two groups was followed up to calculate the median survival time. Recent evaluation criteria were divided into disease progression (PD), partial response (PR), complete remission (CR), and stable condition (SD). RR = (CR + PR)/all cases ×100%. Disease control rate = (CR + PR + SD)/all cases ×100%. The progression-free survival was defined as the time from treatment to disease progression.

Immune index

Peripheral blood was extracted before and after treatment to detect CD3$^+$ (Human CD3D ELISA Kit (CLIA) - LS-F29277, LifeSpan BioSciences, Inc.), CD4$^+$ (Human CD4 ELISA Kit (Sandwich ELISA) - LS-F6263, LifeSpan BioSciences, Inc.) and CD8$^+$ (Human CD8 ELISA Kit (Sandwich ELISA) - LS-F22885, LifeSpan BioSciences, Inc.) using commercial ELISA kits according to manufacturer's instructions.

Adverse reaction

The adverse reaction during the chemotherapy was observed and graded into I-IV according to WHO [8].

Statistical analysis

All data were analyzed by SPSS 19.0 software. The measurement data were presented as mean ± standard deviation (SD) and compared by student t test, while the enumeration data were compared by chi-square test. $P < 0.05$ was considered as statistical significance.

Table 1. Overall effective rate, disease control rate, and median survival time comparison

Group	Overall effective rate/%	Disease control rate/%	Median survival time/month
Control (n = 100)	27	51	8
Observation group (n = 127)	41	83	10
P	0.378	0.036	0.02

Table 2. Lymphocytes changes comparison (x ± s, $g{\cdot}L^{-1}$)

Group	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
Control				
Before treatment	60.37 ± 6.48	27.13 ± 5.06	23.92 ± 5.09	0.93 ± 1.12
After treatment	62.93 ± 5.87	34.02 ± 4.26*	24.03 ± 4.81	1.28 ± 0.22*
Observation group				
Before treatment	61.26 ± 6.03	28.05 ± 4.72	23.40 ± 5.28	0.93 ± 1.06
After treatment	62.84 ± 5.27	39.33 ± 4.21*,#	25.81 ± 4.92	1.48 ± 0.42*,#

*P < 0.05, compared with before treatment in the same group; #P < 0.05, compared with after treatment in the control group.

Results

Short-term and long-term curative effect comparison

Among 100 patients in control group, 3 cases were completely relieved, 24 were partially relieved, 24 were stable, and 49 were progressive. The overall response rate was 27% and the median survival time was 8 months. Of the 127 patients in observation group, 5 cases were completely relieved, 47 cases were partially relieved, 53 cases were stable, and 22 cases were progress. The overall response rate was 41% and the median survival time was 10 months. The overall response rate showed no significant difference between the two groups (P > 0.05), whereas the disease control rate and median survival time in observation group were obviously higher than those in control group (P < 0.05) (**Table 1**).

Immune index comparison

There was no significant difference between the two groups before treatment (P > 0.05). $CD3^+$ showed no obvious change before and after treatment (P > 0.05). $CD4^+$ and $CD4^+/CD8^+$ proportion in both groups were significantly increased after treatment (P < 0.05), while there was no statistical difference in $CD8^+$ between the two groups (P > 0.05). Compared with control group, $CD4^+$ and $CD4^+/CD8^+$ proportion were significantly increased in observation group after treatment (P < 0.05) (**Table 2**; Supplementary Table 1).

Adverse reaction comparison

The adverse reactions in observation group were all in degree I-III, which did not affect the treatment. In control group, 3 cases suffered from degree IV alopecia and were relieved after symptomatic treatment. The others were in degree I-III. The incidence of adverse reactions in observation group was markedly lower than that in control group except nausea, vomiting, and liver damage (P < 0.05) (**Table 3**).

Curative effect comparison

The mean duration of chemotherapy was 3.3 months in observation group and 3.1 months in control group (P = 0.037). The partial remission rates were 47.2% and 43.0%, whereas the disease control rates were 87.4% and 81.0% in observation group and control group, respectively (**Table 4**).

Overall survival rate

There was no significant difference between the two groups in median overall survival (observation group: 7.3 months, control group: 7.0 months, P = 0.63), one-year survival rate (observation group: 34%, control group: 31%), survival in patients with PS 0-1 (observation group: 8.7 months, control group: 7.7 month, P = 0.51), and survival in patients with PS2 (observation group: 4.3 months, control group: 5.1 month, P = 0.54) (**Figure 1**).

Discussion

The occurrence and development of gastrointestinal cancer is a multi-step, multi-stage and orderly process, with the involvement of several genetic factors and epigenetic interactions. It mainly involves the activation of a large number of oncogenes, mutations in tumor suppressor

Table 3. Adverse reaction comparison

Group	Leukopenia	Thrombocytopenia	Nausea and vomiting	Liver damage	Alopecia	Neurotoxicity	Hand-foot syndrome
Control (n = 100)							
0	10	5	0	0	5	8	6
I	16	4	15	5	4	6	7
II	9	10	9	2	30	4	5
III	20	9	18	8	23	21	9
IV	0	0	0	0	3	0	0
Total (case, %)	55 (55.0)	28 (28.0)	42 (42.0)	15 (15.0)	62 (62.0)	40 (40.0)	27 (27.0)
Observation group (n = 127)							
0	12	5	4	2	11	6	5
I	8	6	17	5	19	10	5
II	10	5	14	5	10	8	4
III	6	1	7	2	8	2	0
IV	0	0	0	0	0	0	0
Total (case, %)	36 (28.3)*	17 (13.4)*	42 (33.1)	13 (10.2)	48 (37.8)*	26 (20.5)*	14 (11.0)*

*$P < 0.05$, compared with control.

Table 4. Short-term curative effect comparison

Curative effect	Observation group (n = 127)	Control group (n = 100)
PR	60 (47.2%)	43 (43.0%)
SD	45 (35.4%)	37 (37.0%)
CR	6 (4.7%)	1 (1.0%)
PD	16 (12.6%)	19 (19.0%)

genes and repair genes. Thus, targeting single gene therapy often cannot effectively inhibit tumor growth [9]. Tumors are often associated with decreased genome methylation and certain gene CpG island hypermethylation. Hypermethylation of DNA in local promoter regions, especially CpG islands, leads to tumor growth, differentiation, invasion, and metastasis. It is related to the silence of tumor suppression genes, pro-apoptotic genes, immunoreactive genes, and chemosensitive genes, resulting in tumorigenesis, progression, and resistance to conventional chemotherapies. Epigenetic inhibitors, such as methylation inhibitors, can play a role in the entire genome to restore the expression of multiple tumor suppressor genes and then plays a pedigree to improve the stability of the genome and achieve better results in clinical treatment [10]. Numerous studies suggested that demethylation drugs may change the relevant signaling pathways and sensitize tumors to chemotherapeutic drugs. Therefore, comprehensive treatment programs based on epigenetic drugs may provide a new approach for the treatment of gastrointestinal cancer [11].

In this study, we initially conducted one-arm clinical trial of refractory gastric cancer to evaluate the efficacy and safety of decitabine. From August 2012, our hospital initiated the clinical trial of low-dose decitabine combined with chemotherapy in the treatment of recurrent and refractory gastrointestinal cancer. In this small sample size study, the disease control rate of gastrointestinal cancer reached 80%. Decitabine inhibits DNA double-strand to exert its effect on DNA methylation-activated silencing [12]. Nishikawa K showed that tumor suppressor gene methylation was not only involved in the regulation of the cell cycle and cell apoptosis, but also was related to the sensitivity of chemotherapy drugs. The main pharmacological effect of decitabine is to directly incorporate into DNA through phosphorylation to inhibit DNA methylation transferase, resulting in DNA hypomethylation and cell differentiation or apoptosis [13]. Amaral L reported that decitabine achieved good therapeutic effect in MDS, CML, and other hematological malignancies [14]. Our results revealed that the overall response rate was 27% and median survival time was 8 months in control group, while it was 41% and 10 months in observation group. The disease control rate and median survival time in observation group were significantly higher than those in control group. Miura K adopted decitabine in hematological malignan-

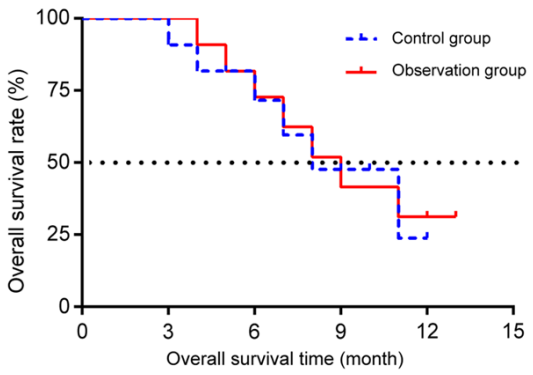

Figure 1. Overall survival rate.

cies and found the five-year survival reached 50% in gastric cancer. However, its application prospect in solid tumor therapy has not been fully explored [15].

In 2012, PODLAHA O explored the correlation between high-dose decitabine and maximum-tolerated measurements in early clinical trials. Unfortunately, the high dose exhibited strong cytotoxicity and bone marrow transplants, and the effects showed hysteresis and dose-dependency [16]. The adverse reactions in observation group were all in degree I-III, which did not affect the treatment. In the control group, 3 cases suffered from degree IV alopecia and were relieved after symptomatic treatment. The others were in degree I-III. The incidence of adverse reactions in the observation group was markedly lower than that in the control except nausea, vomiting, and liver damage. It indicated that the two drugs combined chemotherapy can significantly reduce the incidence of adverse reactions. Preclinical experiments published by PIETRAS demonstrated that decitabine achieved better biological effects at low doses [17]. Low-dose decitabine inhibited DNMT activity without inducing cell death. Maeda S also indicated that decitabine can activate the expression of tumor suppressor genes, cell cycle regulatory genes, and other silencing genes to promote tumor cell apoptosis or inhibit cell proliferation. Clinical trials have revealed that cancer patients exhibited better prognosis and lower toxicity after receiving low-dose decitabine [18].

Currently, decitabine showed demethylation at low concentrations [19]. At present, SHIBATA D confirmed that the it is safe and can activate the expression of tumor suppressor genes, cell cycle regulatory genes, and other silencing genes to promote tumor cell apoptosis or inhibit cell proliferation, thereby increasing the sensitivity to chemotherapeutic drugs [20]. Among them, the study on the mechanism of drug resistance (platinum, fluorouracil, etc.) confirmed that there are several genes involved in abnormal DNA methylation. Tegafur Gimeracil Oteracil Potassium Capsule is a new generation of fluorouracil derivatives with the main components of tegafur, gibberell, and oteracil potassium. Olipracil potassium can specifically reduce the stomach intestinal reaction induced by Tegafur. It was reported that Tegafur Gimeracil Oteracil Potassium Capsule can improve efficiency and control rate, and reduce the incidence of adverse reactions [21]. Compared with cisplatin, it has stronger anticancer activity and less adverse reactions, and is currently used as a gastric cancer chemotherapeutic drug [22]. $CD4^+$ and $CD4^+/CD8^+$ proportion in both groups were significantly increased after treatment ($P < 0.05$), while there was no statistical difference in $CD8^+$ between the two groups ($P > 0.05$). Compared with control, $CD4^+$ and $CD4^+/CD8^+$ proportion were significantly increased in observation group after treatment, indicating that both of the chemotherapy regimens can enhance the immune function of patients, and the two drugs combined effect was better. It was found that multiple drug combination chemotherapy can improve the effect of chemotherapy, whereas increase the incidence of chemotherapy-related toxic reactions. Therefore, a reasonable combination of chemotherapy drugs and minimize toxicity is currently the research hot spots of gastric cancer [23].

Conclusion

Low-dose decitabine combined with chemotherapy can improve the clinical efficacy, enhance immune function, while reduce the incidence of adverse reactions in the patients with refractory gastric cancer.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] McGuire S. World cancer report 2014. Geneva, Switzerland: world health organization, international agency for research on cancer, WHO Press, 2015. Adv Nutr 2016; 7: 418-419.

[2] Park SC and Chun HJ. Chemotherapy for advanced gastric cancer: review and update of current practices. Gut Liver 2013; 7: 385-393.

[3] Wang X, Wang ML, Zhou LY, Lu XY, Yang JF and Yu HG. Randomized phase II study comparing paclitaxel with S-1 vs. S-1 as first-line treatment in patients with advanced gastric cancer. Clin Transl Oncol 2013; 15: 836-842.

[4] Unek IT, Unek T, Oztop I, Akman T, Atilla K, Ellidokuz H, Bora S, Sarioglu S and Yilmaz U. Bimonthly regimen of high-dose leucovorin, infusional 5-fluorouracil, epirubicin and cisplatin (modified ECF) as adjuvant chemotherapy in resected gastric adenocarcinoma. Chemotherapy 2012; 58: 233-240.

[5] Chen S, Feng X, Li Y, Yuan X, Zhou Z and Chen Y. Efficacy and safety of XELOX and FOLFOX6 adjuvant chemotherapy following radical total gastrectomy. Oncol Lett 2012; 3: 781-786.

[6] Nishina T, Kato T, Yamazaki K, Yoshino T, Miyata Y, Esaki T, Moriwaki T, Boku N and Hyodo I. A phase II study of S-1, oxaliplatin, oral leucovorin, and bevacizumab combination therapy (SOLA) in patients with unresectable metastatic colorectal cancer. Cancer Chemother Pharmacol 2015; 76: 547-553.

[7] Xu R, Ma N, Wang F, Ma L, Chen R, Chen R, Kebinu M, Ma L, Han Z, Ayixiamu, Mayier M, Su P, Naman Y, Jieensi H, Yang H, Adili A, Aili S and Liu J. Results of a randomized and controlled clinical trial evaluating the efficacy and safety of combination therapy with Endostar and S-1 combined with oxaliplatin in advanced gastric cancer. Onco Targets Ther 2013; 6: 925-929.

[8] Skulsky SL, O'Sullivan B, McArdle O, Leader M, Roche M, Conlon PJ and O'Neill JP. Review of high-risk features of cutaneous squamous cell carcinoma and discrepancies between the American joint committee on cancer and NCCN clinical practice guidelines in oncology. Head Neck 2017; 39: 578-594.

[9] Lee JH, Kim SH, Oh SY, Lee S, Lee H, Lee HJ and Kim HJ. Third-line docetaxel chemotherapy for recurrent and metastatic gastric cancer. Korean J Intern Med 2013; 28: 314-321.

[10] Kunisaki C, Takahashi M, Makino H, Oshima T, Fujii S, Takagawa R, Kimura J, Kosaka T, Ono HA, Akiyama H, Kameda K, Kito F, Morita S and Endo I. Phase II study of biweekly docetaxel and S-1 combination chemotherapy as first-line treatment for advanced gastric cancer. Cancer Chemother Pharmacol 2011; 67: 1363-1368.

[11] Oh SY, Kwon HC, Jeong SH, Joo YT, Lee YJ, Cho S, Kang MH, Go SI, Lee GW, Kim H and Kang JH. A phase II study of S-1 and oxaliplatin (SOx) combination chemotherapy as a first-line therapy for patients with advanced gastric cancer. Invest New Drugs 2012; 30: 350-356.

[12] Miyamoto Y, Tsuji A, Tanioka H, Maekawa S, Kawanaka H, Kitazono M, Oki E, Emi Y, Murakami H, Ogata Y, Saeki H, Shimokawa M, Natsugoe S, Akagi Y, Baba H and Maehara Y. S-1 and irinotecan plus bevacizumab as second-line chemotherapy for patients with oxaliplatin-refractory metastatic colorectal cancer: a multicenter phase II study in Japan (KSCC1102). Int J Clin Oncol 2016; 21: 705-712.

[13] Nishikawa K, Yamada Y, Ishido K, Gotoh M, Bando H, Sugimoto N, Nishina T, Amagai K, Chin K, Niwa Y, Tsuji A, Imamura H, Tsuda M, Yasui H, Fujii H, Yamaguchi K, Yasui H, Hironaka S, Shimada K, Miwa H, Hamada C and Hyodo I. Impact of progression type on overall survival in patients with advanced gastric cancer based on randomized phase III study of S-1 plus oxaliplatin versus S-1 plus cisplatin. Gastric Cancer 2017; 20: 640-645.

[14] Amaral L, Mendes J, Martins P, Bernardo L, Quintanilha R, Santos V and Melo AS. Survival benefits of palliative gastric cancer resection - a regional center experience. Hepatogastroenterology 2012; 59: 1651-1656.

[15] Miura K, Shirasaka T, Yamaue H and Sasaki I. S-1 as a core anticancer fluoropyrimidine agent. Expert Opin Drug Deliv 2012; 9: 273-286.

[16] Podlaha O, Riester M, De S and Michor F. Evolution of the cancer genome. Trends Genet 2012; 28: 155-163.

[17] Pietras A. Cancer stem cells in tumor heterogeneity. Adv Cancer Res 2011; 112: 255-281.

[18] Maeda S, Wada H, Tanemura M, Kobayashi S, Kawamoto K, Marubashi S, Eguchi H, Umeshita K, Doki Y, Mori M and Nagano H. [A case of hepatocellular carcinoma treated by transcatheter arterial chemoembolization and hepatectomy after subtotal stomach preserving pancreaticoduodenectomy for duodenal cancer]. Gan To Kagaku Ryoho 2012; 39: 1988-1990.

[19] Makni A, Chebbi F, Azzouz H, Magherbi H, Jouini M, Kacem M and Safta ZB. A case of primary

malignant fibrous histiocytoma of the duodenum. Int J Surg Case Rep 2011; 2: 103-105.
[20] Shibata D. Cancer. Heterogeneity and tumor history. Science 2012; 336: 304-305.
[21] Zhao Y, Guo Q, Chen J, Hu J, Wang S and Sun Y. Role of long non-coding RNA HULC in cell proliferation, apoptosis and tumor metastasis of gastric cancer: a clinical and in vitro investigation. Oncol Rep 2014; 31: 358-364.
[22] Mei D, Song H, Wang K, Lou Y, Sun W, Liu Z, Ding X and Guo J. Up-regulation of SUMO1 pseudogene 3 (SUMO1P3) in gastric cancer and its clinical association. Med Oncol 2013; 30: 709.
[23] Yang F, Xue X, Bi J, Zheng L, Zhi K, Gu Y and Fang G. Long noncoding RNA CCAT1, which could be activated by c-Myc, promotes the progression of gastric carcinoma. J Cancer Res Clin Oncol 2013; 139: 437-445.

Supplementary Table 1. The changes of CD3, CD4 and CD8 cells before and after treatment in individuals

	Efore treatment				Fter treatment			
	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$
1	56.85	22.70	30.08	0.53	64.20	44.83	17.87	1.35
2	63.85	28.34	19.59	1.57	58.45	44.14	33.26	0.74
3	58.44	32.72	20.57	1.56	59.99	42.73	27.08	1.63
4	55.54	29.43	15.50	1.02	70.74	35.53	21.70	1.70
5	63.42	36.28	28.95	1.24	75.49	36.14	25.80	1.60
6	57.72	34.82	28.02	0.57	58.98	49.45	20.18	1.01
7	59.61	27.83	25.60	0.43	66.28	43.98	29.13	1.85
8	73.16	29.37	22.87	0.99	63.74	41.20	28.00	1.50
9	69.16	33.27	32.26	1.84	60.93	40.57	19.56	1.19
10	56.51	28.71	22.64	0.26	65.35	36.69	38.78	1.81
11	52.82	27.60	25.52	1.01	64.94	40.91	33.56	1.57
12	58.44	25.19	26.11	1.08	59.95	41.33	21.22	1.63
13	69.66	26.55	29.12	0.54	54.15	32.19	31.77	1.42
14	56.41	29.17	14.76	1.37	59.83	30.16	20.24	1.65
15	60.82	27.81	20.97	1.49	52.56	30.59	33.87	1.11
16	62.65	26.02	27.69	0.01	54.96	39.50	23.66	1.38
17	53.18	25.82	24.66	1.02	68.05	36.46	28.60	1.82
18	66.75	36.84	29.14	0.66	59.20	40.73	23.18	1.53
19	50.15	37.42	24.89	0.89	57.06	39.47	31.91	2.25
20	60.72	25.13	23.18	0.61	67.59	48.18	34.30	2.39
21	63.26	26.50	25.93	0.16	57.46	33.64	18.49	1.58
22	56.37	33.24	24.53	1.27	58.28	34.53	28.73	1.73
23	59.35	25.14	10.86	1.52	68.73	42.28	26.77	0.80
24	57.63	18.96	33.32	0.53	56.40	41.13	28.52	1.63
25	62.15	27.66	15.04	1.74	57.10	44.73	22.95	1.57
26	65.23	29.33	21.59	0.91	64.47	42.11	16.74	2.13
27	63.14	28.04	27.68	0.11	62.61	43.09	29.57	2.32
28	61.28	25.78	27.43	0.27	62.18	39.90	30.73	2.30
29	59.22	29.33	24.33	1.36	71.20	41.81	23.98	1.87
30	63.42	26.34	28.21	0.17	64.52	44.02	24.61	1.73
31	62.41	23.89	15.19	0.43	63.26	39.70	31.64	1.32
32	69.66	20.14	20.26	2.10	57.50	40.05	31.97	0.55
33	64.82	22.07	24.62	0.52	57.84	43.38	17.09	1.12
34	60.73	28.16	30.38	1.14	58.67	32.13	35.66	1.09
35	58.34	30.26	19.30	0.46	54.30	40.70	30.15	1.06
36	59.79	23.25	29.44	1.27	61.71	30.93	26.78	1.44
37	54.33	31.78	23.90	0.64	68.43	39.15	23.32	0.98
38	66.17	23.85	32.73	0.85	73.42	38.26	16.97	1.97
39	59.64	24.72	14.47	1.63	65.59	39.81	27.10	1.63
40	73.81	26.82	22.45	2.03	63.02	39.93	27.37	1.09
41	70.39	24.12	27.24	1.38	69.87	36.59	32.30	1.95
42	65.37	31.65	24.92	1.17	61.24	35.97	36.77	0.90
43	58.01	22.34	11.94	0.27	72.63	44.66	26.87	1.29
44	53.42	25.89	30.11	1.75	66.78	39.82	32.52	0.97
45	63.73	26.20	26.91	0.66	61.82	45.66	20.20	1.18
46	66.12	33.47	31.12	0.48	62.59	45.46	28.19	1.91

47	57.71	25.64	13.70	0.69	58.09	39.34	26.12	1.27
48	63.95	20.63	27.34	0.33	59.01	39.87	27.45	1.85
49	69.58	35.32	29.96	1.58	66.26	36.82	25.09	1.86
50	59.75	28.75	23.55	1.08	60.69	38.41	20.26	1.20
51	58.23	26.48	19.52	1.57	61.25	42.35	28.12	1.02
52	72.18	20.22	20.75	0.77	64.46	43.13	29.10	1.66
53	73.54	31.84	34.88	0.69	65.66	41.03	21.40	1.76
54	54.30	26.99	29.58	0.45	70.23	41.81	17.73	1.38
55	56.05	40.56	34.92	0.92	66.59	39.57	20.67	1.50
56	65.68	27.41	16.58	1.10	64.22	37.85	27.24	0.66
57	58.19	25.57	24.39	1.25	72.72	45.17	32.76	0.93
58	62.44	23.49	29.36	1.42	65.90	34.69	25.05	1.27
59	62.93	29.98	28.00	0.80	68.70	30.75	24.67	1.23
60	73.39	25.11	12.78	0.89	69.75	41.43	32.73	1.38
61	71.79	29.21	20.30	1.32	67.87	37.88	27.77	1.80
62	66.69	27.18	21.76	1.38	73.68	42.74	26.18	1.27
63	54.87	22.71	20.28	0.86	56.84	35.14	25.75	1.63
64	51.82	39.74	16.02	0.81	59.70	40.89	30.73	1.90
65	58.69	26.28	21.82	1.08	61.68	37.41	26.40	1.08
66	66.15	25.75	18.61	0.97	65.53	41.07	23.10	1.25
67	51.31	22.37	25.49	0.96	71.35	41.50	27.98	1.25
68	60.45	31.55	18.50	0.05	68.66	38.76	27.28	1.68
69	52.74	32.43	25.20	0.09	65.35	34.81	23.08	1.32
70	56.18	27.98	18.51	0.75	58.83	35.07	32.70	1.14
71	66.74	32.37	21.25	1.92	73.26	39.70	16.74	1.39
72	59.86	33.07	21.33	1.10	52.84	38.84	24.15	1.84
73	63.29	22.49	25.57	0.35	57.44	32.34	29.69	0.87
74	61.15	27.73	26.99	0.48	67.21	35.23	24.73	1.11
75	57.78	30.56	29.00	0.07	66.07	42.37	33.85	0.58
76	59.74	27.76	23.40	0.28	56.01	41.01	24.73	1.58
77	57.78	25.15	30.05	1.85	65.55	42.87	23.41	1.36
78	58.69	28.67	25.14	0.79	65.35	42.41	28.68	1.80
79	59.00	24.20	22.60	0.38	58.61	40.56	28.75	1.74
80	60.51	31.18	18.31	0.68	57.12	38.72	31.75	1.70
81	54.86	21.25	22.64	1.11	61.40	39.08	30.13	1.08
82	58.02	26.55	30.96	0.85	60.18	38.75	25.91	1.57
83	53.28	31.38	10.77	0.00	65.39	47.23	24.81	1.62
84	62.69	32.31	20.93	1.77	59.25	41.45	32.82	1.83
85	72.54	25.69	26.88	0.12	66.90	38.00	25.07	1.20
86	50.74	27.55	26.47	0.31	55.70	31.90	19.12	1.98
87	56.94	32.05	24.09	0.78	59.73	40.42	21.79	2.18
88	69.08	33.80	27.31	0.25	61.34	41.28	21.61	1.44
89	55.97	25.35	23.62	0.88	62.16	38.08	19.52	1.57
90	66.91	29.40	31.17	0.85	60.05	36.60	25.94	1.39
91	67.78	23.16	25.77	0.68	63.29	42.72	21.50	1.36
92	54.49	25.11	18.79	1.50	60.49	43.95	19.34	1.39
93	53.85	25.86	27.88	0.62	61.08	42.04	16.06	0.68
94	62.08	30.05	26.65	2.16	65.72	28.30	24.46	2.13
95	58.43	27.52	22.11	0.41	64.39	38.88	19.10	2.17
96	61.40	22.07	20.45	0.39	65.46	33.45	23.32	0.98

97	63.37	23.15	24.56	1.54	68.53	40.47	20.42	1.19
98	56.01	26.63	21.48	0.55	60.02	37.13	25.36	1.93
99	54.59	20.36	32.64	1.49	59.60	39.49	27.90	1.98
100	64.85	37.85	17.92	1.33	64.97	35.11	19.87	0.94
101	60.94	31.49	29.31	0.93	64.25	37.52	23.51	1.28
102	66.73	17.82	23.70	0.92	67.86	34.12	24.74	1.33
103	72.22	36.49	24.76	0.60	59.21	39.77	24.19	1.74
104	62.89	17.05	20.89	0.04	60.80	39.24	20.77	2.10
105	56.11	31.98	24.48	0.98	64.59	43.62	22.29	1.48
106	53.02	26.20	19.95	0.91	63.64	42.93	16.26	1.37
107	58.87	26.46	19.53	0.78	60.12	38.90	16.31	0.70
108	62.32	28.86	24.61	0.46	61.89	40.71	33.57	1.16
109	49.13	33.44	20.53	0.76	64.63	31.73	31.72	1.78
110	59.57	30.23	22.69	1.70	68.35	40.52	20.91	1.00
111	58.40	25.33	30.56	1.68	62.50	38.12	15.22	1.70
112	58.35	26.43	17.07	0.21	70.74	37.84	33.89	1.61
113	67.69	21.22	15.88	1.04	64.03	40.30	26.00	1.46
114	61.30	29.60	28.89	1.37	77.03	42.23	27.04	2.01
115	63.38	31.39	31.66	0.78	66.11	36.96	31.14	1.61
116	67.58	37.92	27.37	1.91	69.57	38.19	25.54	1.02
117	58.84	35.89	22.84	0.73	52.60	34.90	19.38	0.92
118	73.40	23.92	29.51	0.87	50.64	40.70	23.25	1.50
119	64.34	26.63	25.44	0.21	66.02	40.33	27.09	1.49
120	59.01	28.25	22.26	0.74	66.91	33.22	20.92	1.74
121	60.19	23.69	28.90	0.84	69.04	35.34	34.34	2.04
122	53.18	34.46	29.98	1.44	65.49	42.13	18.87	2.09
123	66.79	25.74	24.50	1.34	66.17	39.02	31.67	1.49
124	68.25	28.57	32.17	0.89	58.57	36.73	23.73	1.63
125	58.43	26.10	21.93	1.11	60.65	39.38	29.82	2.01
126	62.79	29.71	21.71	0.27	65.94	42.65	22.39	2.09
127	53.90	30.74	18.93	0.22	62.88	43.58	20.46	1.59
	61.12047244	27.94504	24.05197	0.90188976	63.32614173	39.2974	25.70528	1.48858268
	5.725115769	4.580159	5.238332	0.52146274	5.137098966	3.914425	5.215894	0.40491372