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Original Article

Safety and efficacy of Compound Huangdai Tablets combined with all-trans retinoic acid for treatment of acute promyelocytic leukemia: Clinical evidence and potential mechanisms

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ABSTRACT

Objective: To evaluate the safety and efficacy of Compound Huangdai Tablets (*Realgar-Indigo Naturalis* formula, RIF) combined with all-*trans* retinoic acid (ATRA) to treat acute promyelocytic leukemia (APL).
Methods: This study was registered in PROSPERO (CRD42018108118). The relevant literatures on RIF treatment of APL were systematically searched in the following databases: China National Knowledge Infrastructure, Wanfang, VIP Medical Information System, Chinese Biomedical Database, EMBASE, Cochrane Library, and PubMed. The quality of the included studies was evaluated and Review Manager 5.3 software and Stata 13.0 software were used to perform the Meta-analysis. In addition, this study used the method of network pharmacology to conduct a preliminary exploration of the mechanism of RIF on APL.
Results: The study included 12 studies involving 775 APL patients. The Meta-analysis showed that there was no significant difference ($P > 0.05$) between the RIF group and the arsenic trioxide (ATO) group for primary outcomes, secondary outcomes apart from liver dysfunction. The incidence of liver dysfunction ($P = 0.006$) in the RIF group were significantly lower than those in the ATO group. In addition, the cost of maintenance therapy in the RIF group was significantly lower ($P < 0.05$) than the ATO group. Besides, the active ingredients in RIF mainly act on targets proteins such as ACHE, NCOA2, RXRA, and then play a role in the treatment of APL through regulating multiple molecular mechanisms, such as TP53 regulates transcription of cell cycle genes, nuclear receptor transcription pathway.
Conclusion: There was no significant difference in efficacy of oral RIF combined with ATRA compared with intravenous ATO combined with ATRA for the treatment of APL. The oral RIF exposed patients to less risk, offered more convenience and had lower prices. RIF can treat APL by multi-target and multi-pathway interventions that inducing apoptosis of APL cells and inhibiting the proliferation of APL cells, and so on. Therefore, oral RIF in the treatment of APL is worthy of further research and development.

1. Introduction

Acute promyelocytic leukemia (APL) is the promyelocytic M_3 subtype of acute myeloid leukemia (AML), and in most patients, it is characterized by the presence of a PML-RARα fusion gene formed by chromosomal translocation t(15; 17) (Adams & Nassiri, 2015; Braess, 2016) Cases of APL account for approximately 5%–15% of AML, and there are approximately 600–800 new patients per year in the United States (Giri et al., 2017; Ribeiro & Rego, 2006). The vast majority of patients diagnosed with APL for the first time are young and middle-aged individuals between the ages of 20 and 50 (Giri, et al., 2017; Ribeiro & Rego, 2006). Clinically, patients with APL have a high incidence of early hemorrhage, with most manifesting as disseminated intravascular coagulation, resulting in high early mortality (Ribeiro & Rego, 2006; NCCN, 2020). At present, APL is mainly treated with intravenous arsenic trioxide (ATO), all-*trans* retinoic acid (ATRA) and chemotherapy drugs (Blackburn et al., 2016; Rao et al., 2013). However, the chemotherapy drugs can lead to an increased ten-

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dency to bleed, leading to increase in early mortality (Wang & Chen, 2008; Bernard et al., 1973). And then, although ATRA has a significant therapeutic effect on APL, taking ATRA can cause fatal differentiation syndrome, and patients treated with ATRA are prone to relapse and develop resistance (Wang & Chen, 2008; Chen & Chen, 2017). Therefore, ATRA is usually combined with other drugs to achieve better therapeutic effect. Finally, although ATO is currently the first-line drug for the treatment of APL, there are also problems such as fatal differentiation syndrome, poor patient compliance, short drug shelf life (Zhu & Huang, 2014; Kumana & Kuang, 2006). Thus, further searches for drugs that are more convenient for clinical application has practical clinical value.

Interestingly, clinical pharmacokinetic researchers have found that oral arsenic can achieve almost the same bioavailability as intravenous arsenic (Kumana et al., 2002). At the same time, the cost of treating patients with oral arsenic is significantly lower than that of first-line therapy with intravenous arsenic, which can reduce the financial burden on patients (Jiang et al., 2015). Compound Huangdai Tablet (*Realgar-Indigo Naturalis* formula, RIF) is an oral Chinese herbal compound preparation for the treatment of APL, developed by the prominent Chinese physician professor Shi-lin Huang (Xu et al., 2017). It consists of four traditional Chinese medicines: *Realgar*, *Indigo Naturalis*, *Salvia miltiorrhiza*, and *Pseudostellaria heterophylla* (Xu et al., 2017). Basic research on RIF shows that the main active components of RIF are As_2S_2 (A), tanshinone IIA (T) and indirubin (I) (Wang, et al., 2008). The combination of the above three components, can promote the absorption of arsenic by cells, enhance the degradation of the APL oncoprotein, enhance the differentiation of APL cells, inhibit the division of APL cancer cells, and thereby enhance the therapeutic effect (Wang, et al., 2008; Zhang, et al., 2018; Hu, et al., 2010). In clinical studies, RIF combined with ATRA is effective and safe. For example, Xiao-jun Huang's research showed that only two oral drugs (RIF and ATRA) can allow patients to achieve complete remission (CR) and patient quality of life can be close to that of healthy people (Zhu & Huang, 2014). RIF combined with ATRA has been the first-line treatment of low/intermediate-risk APL, which was included in the Chinese guidelines for diagnosis and treatment of APL (version 2018), developed by the hematology branch of Chinese Medical Association (The hematology branch of Chinese Medical Association, 2018).

However, there is still a lack of systematic review studies of the efficacy and safety of RIF combined with ATRA, and the potential targets of RIF acting on APL are still unclear. In this study, we conducted a Meta-analysis of the effectiveness and safety of RIF combined with ATRA in the treatment of APL, and network pharmacology was utilized to further explain the mechanism of RIF in the treatment of APL. Based on the above analysis results, we aim to provide a more valid basis for the clinical application of RIF in the treatment of APL.

2. Methods

2.1. Meta-analysis to evaluate treatment safety and efficacy of RIF combined with ATRA on APL

2.1.1. Search strategy

The following research databases were searched: China National Knowledge Infrastructure (CNKI), Wanfang, VIP Medicine Information System (VIP), Chinese Biomedical Database (CBM), EMBASE, Cochrane Library, and PubMed. The date of the literature retrieval was from the establishment of the different databases until February 10, 2021. The initial search included the following keywords/phrases: "huangdai", "qingdai", "baixuekang", "realgar indigo", "RIF", and "CRNIT" (Title/abstract) and "leukemia", "hematologic malignancy", and "blood cancer" (Title/abstract). The retrieval results were preliminarily screened by exporting Excel tables from the databases, and the remaining documents were downloaded in full for further screening.

2.1.2. Inclusion criteria

The inclusion criteria were as follows: (1) Literatures were described as clinical randomized controlled trials (RCTs) or quasi-randomized control trials (qRCTs); (2) Patients with APL who met specific diagnostic criteria; (3) The treatment plan of the experimental group was based on oral RIF combined with ATRA (hereinafter referred to as the RIF group) while the control group was based on intravenous ATO combined with ATRA (hereinafter referred to as the ATO group); If there was a CT regimen in the protocol, it must have been the same for both the experimental and control groups; And (4) there was no language restriction for articles.

2.1.3. Exclusion criteria

The exclusion criteria of this study were as follows: (1) duplicated literatures; (2) experimental studies or reviews; (3) The baseline between the experimental group and the control group was not equal; (4) Literature that unable to extract outcomes.

2.1.4. Data extraction

Two reviewers (Qian-qian Huang / Tao Wang) independently extracted information regarding general information, diagnostic criteria, dosing regimen, outcomes and adverse reactions reported in the literature. General information included study, publication year, and sample size of the experimental group and control group. Outcomes included primary outcomes (complete remission rate, relapse rate, mortality, CR time, 2-year disease-free survival (DFS)) and secondary outcomes (peripheral blood (white blood cells (WBC), platelets (PLT)), biochemical indicators (alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), urea (UREA), adverse reactions (liver dysfunction, cardiac abnormalities, differentiation syndrome, hyperleukocytosis, coagulation abnormalities, gastro-intestinal response, and treatment costs). The primary outcome index refers to the indicator that reflects the final endpoint of the patient's treatment and the therapeutic effect. The secondary outcome index refers to the indicator related to the patient's post-treatment symptoms and functional activities. If there is a disagreement, a third reviewer participated in the discussion until a consensus was reached.

2.1.5. Quality assessment

Based on the systematic evaluation bias risk assessment criteria provided by the Cochrane Collaboration Network, this study conducted an objective and rigorous assessment of the quality of the included literatures (Higgins & Green, 2011). The assessment consists of seven main areas: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. Each item was evaluated using three levels: "low risk", "high risk" and "unclear risk". The quality assessment was carried out independently and cross-checked by two reviewers. If there was a disagreement, it was discussed with a third reviewer.

2.1.6. Statistical analysis

RevMan5.3 software provided by the Cochrane Collaboration was utilized for the Meta-analysis. Relative risk (*RR*) was adopted for dichotomous variables, and mean difference (*MD*) was applied for continuous variables. The data analysis provided a 95% confidence interval (95% *CI*).

This study used a chi-square test on the basis of Cochran's Q test and I^2 statistic at the $P < 0.10$ level of significance to judge heterogeneity. If $P \leq 0.1$ and $I^2 > 50\%$, the samples had higher heterogeneity, and a random effect model was used for the Meta-analysis; Otherwise, we concluded that the heterogeneity between samples was small and used a fixed effect model to perform the Meta-analysis. In addition, in order to further demonstrate whether the research results were stable, we adopted a dual mathematical modeling method to test the stability of each research result. A funnel plot was used to assess the publication bias of the complete remission rate of the primary outcome measures and used the Egger's and Begg's tests to statistically determine the publication bias.

2.2. Network pharmacology-based predicting of potential actions of RIF on APL

We searched for the potential active compounds and genes associated with *Realgar*, *Indigo Naturalis*, *S. miltiorrhiza* and *P. heterophylla* in two databases: TCMSP (http://tcmspw.com/tcmsp.php) and BATMAN-TCM (http://bionet.ncpsb.org/batman- tcm/). The screening conditions for the potential active compounds were the oral bioavailability (OB) greater than or equal to 30% and the drug-like index (DL) greater than or equal to 0.18. Next, "acute promyelocytic leukemia" was used as the keyword to collect relevant targets genes related to APL in OMIM (http://www.omim.org/). In addition, the Drug Bank (http://www.drugbank.ca/) database was used to collect the targets of the standard drugs for the treatment of APL recorded in the APL diagnosis and treatment guidelines (NCCN, 2020). The Uniprot database (https://www.uniprot.org/) was used to unify the target data. Based on the potential active compounds of RIF and the cross targets of RIF and APL, we build a "drugs-drug targets-disease targets" network diagram in Cytoscape 3.7.1. And then, on the basis of the value degree, betweenness and closeness, the core targets were screen out. Finally, this study used Reactome for pathway enrichment analysis.

3. Results

3.1. Inclusion of studies

According to the search strategy, a total of 936 articles were retrieved. By reviewing the title and abstract of the literatures, we excluded repeated literature, review literature, and experimental studies. Furthermore, we read the full text of 46 articles and finally included 12 articles (Feng et al., 2010; Wang, 2013; Wei & Zhong, 2013; Zhu et al., 2013; Chen et al., 2014; Yang, 2016; Xie, 2017; Yang et al., 2018; Zhu et al., 2018; Qu et al., 2018; He et al., 2019; Qiao, 2020) (Fig. 1).

3.2. Characteristics of included studies

This study included 12 articles, which included 10 (Feng et al., 2010; Wang, 2013; Wei & Zhong, 2013; Chen et al., 2014; Xie, 2017; Yang et al., 2018; Zhu et al., 2018; Qu et al., 2018; He et al., 2019; Qiao, 2020) RCTs and two (Zhu et al., 2013; Yang, 2016) qRCTs, and all included clinical trial studies were conducted in China (Table 1). A total of 775 patients (400 in the

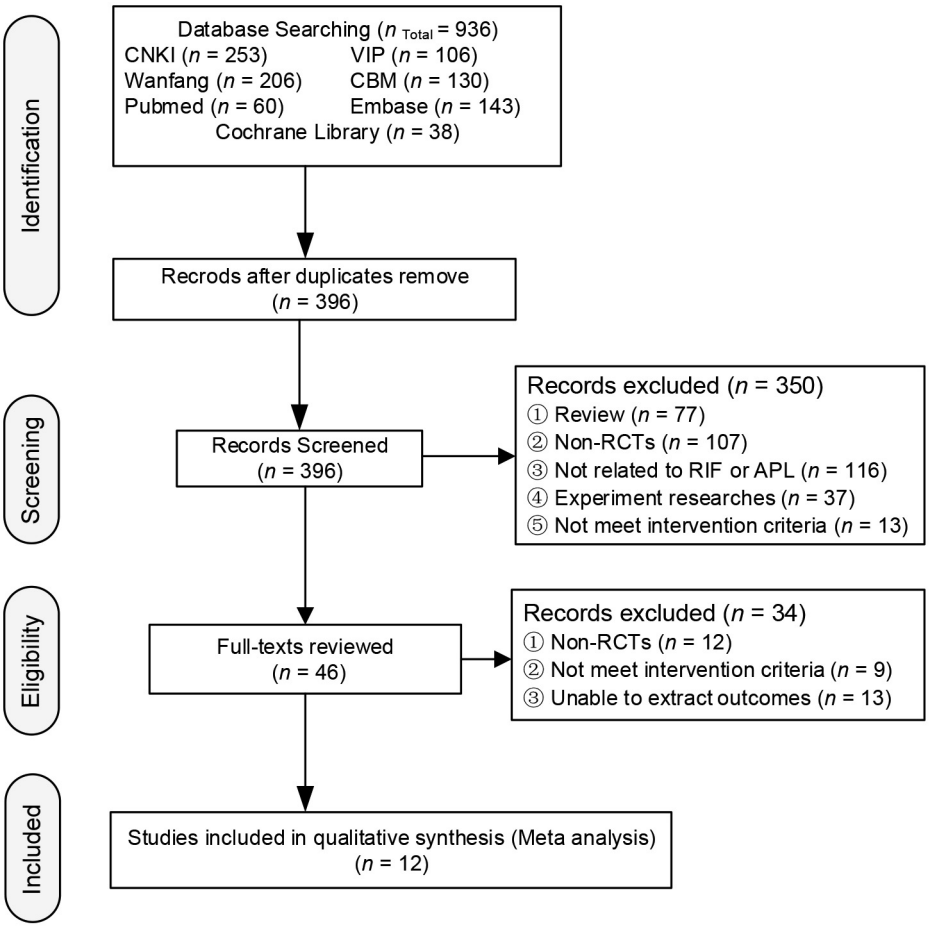

Fig. 1. Flowchart of study selection.

Table 1
Characteristics of included studies.

References	Number of patients (male/female)		Age(year; E/C)	Sanz risk		Intervention		Outcomes	Diagnostic criteria
	E	C		Intermediate-to-low risk(E/C)	High risk (E/C)	E	C		
Feng et al., 2010	11/10	12/9	17–76/16–74	8/7	13/14	RIF(4.05–8.1 g/d, tid) + ATRA + CT	ATO + ATRA + CT	CRR; mortality; WBC; PLT; coagulation abnormalities; liver dysfunction; gastro-intestinal response	Hematology diagnosis and efficacy standards (2007)
Wang, 2013	6/4	5/5	34 ± 5.963/ 33.3 ± 6.36	NA	NA	RIF(4.05 g/d, tid) + ATRA + CT	ATO + ATRA + CT	WBC; PLT; ALT; AST; UA; UREA; treatment costs	Hematology diagnosis and efficacy standards (2007)
Wei & Zhong, 2013	3/5	14	NA	NA	NA	RIF(2.43–8.1 g/d, tid) + ATRA + CT	ATO + ATRA + CT	CRR; CR time; mortality; hyperleukocytosis	FAB criteria
Zhu et al., 2013	61/53	65/52	33(15–60)/39 (15–60)	93/92	21/25	RIF(60 mg/kg.d, qd) + ATRA + CT	ATO + ATRA + CT	CRR; mortality; relapse rate; 2-year DFS; differentiation syndrome	WHO Diagnostic Classification (2008)
Chen et al., 2014	7/8	6/7	NA	15/13	0/0	RIF (NA) + ATRA + CT	ATO + ATRA + CT	CRR; CR time; mortality; relapse rate; liver dysfunction; gastro-intestinal response	FAB criteria
Yang, 2016	7/6	6/6	37.92 ± 6.97/ 35.92 ± 5.76	NA	NA	RIF(1.35–2.7 g/d, tid) + ATRA + CT	ATO + ATRA + CT	WBC; PLT; ALT; AST; UA; UREA ;treatment costs	Hematology diagnosis and efficacy standards (2007)
Xie, 2017	7/7	9/7	39.01 ± 13.06/ 38.84 ± 10.52	11/13	3/3	RIF(2.43–4.05 g/d, tid) + ATRA + CT	ATO + ATRA + CT	CRR; CR time; mortality; WBC; liver dysfunction; cardiac abnormalities; coagulation abnormalities; hyperleukocytosis	Chinese guidelines for diagnosis and treatment of acute promyelocytic leukemia (2014)
Yang, et al., 2018	22/18	29/13	9.9 (2.1–16)/ 7.8 (1–13)	32/28	8/14	RIF(135 mg/kg·d, tid) + ATRA + CT	ATO + ATRA + CT	liver dysfunction;cardiac abnormalities; coagulation abnormalities; differentiation syndrome	FAB criteria
Zhu et al., 2018	33/36	16/20	34 (24–47)/36 (30–46)	69/36	0/0	RIF(60 mg/kg·d, tid) + ATRA	ATO + ATRA	CRR; mortality; relapse rate; 2-year DFS; liver dysfunction; cardiac abnormalities ; differentiation syndrome;hyperleukocytosis	WHO Diagnostic Classification (2008)
Qu et al., 2018	21/10	19/12	34.5 ± 10/ 33 ± 8.75	31/31	0/0	RIF(4.05 g/d, tid) + ATRA + CT	ATO + ATRA + CT	CRR; relapse rate; ALT; AST; UA; UREA; cardiac abnormalities	Chinese guidelines for diagnosis and treatment of acute promyelocytic leukemia (2014)
He et al., 2019	11/9	7/11	37.3(17–68)/ 39 (23–59)	20/18	0/0	RIF(60 mg/ kg·d) + ATRA + CT	ATO + ATRA + CT	CRR; CR time; mortality; treatment costs	Hematology diagnosis and efficacy standards (2007)
Qiao, 2020	24/21	26/19	37.39 ± 6.83/ 36.98 ± 6.73	NA	NA	RIF(8.1 g/d, tid) + ATRA	ATO + ATRA	Mortality; relapse rate; differentiation syndrome; gastro-intestinal response	Chinese guidelines for diagnosis and treatment of acute promyelocytic leukemia (2018)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; ATO = arsenic trioxide; ATRA = all-trans retinoic acid; CR = complete remission; CRR = complete remission rate; CT = Chemotherapy; DFS = disease-free survival; E/C = Experimental group/ Control Group; PLT = platelets; RIF = Realgar-Indigo naturalis formula; UA = uric acid; WBC = white blood cell

experimental group and 375 in the control group) were included in the study. There were no significant differences in age and sex between the groups. The publication dates were from 2010 to 2019. For the literature with a CT regimen, the main CT drugs used were daunorubicin, cytarabine, mitoxantrone, etc., and the CT regimens of the experimental group and the control group were consistent.

3.3. Quality of studies

Among the 12 articles, two studies (Xie, 2017; Qu et al., 2018) were randomized by a random number table method, one literature (Yang et al., 2018) used computer-generated codes to randomly assign patients, one study (Zhu et al., 2013) used a computer-generated random allocation schedule to assign patients, and the random assignment of one study (Zhu et al., 2018) was done by permuted blocks (block size 6) and stratification by trial centre and was implemented through an interactive web response system, while two (Wang, 2013; Yang, 2016) of the studies were qRCTs, in which patients were randomized according to enrollment time and a blinded method was used. The remaining studies did not report using a specific randomization method. In addition, there were two articles (Wei & Zhong, 2013; He et al., 2019) with incomplete outcome data. None of the literature mentions whether allocation concealment had been done or whether they blinded the results (Fig. 2).

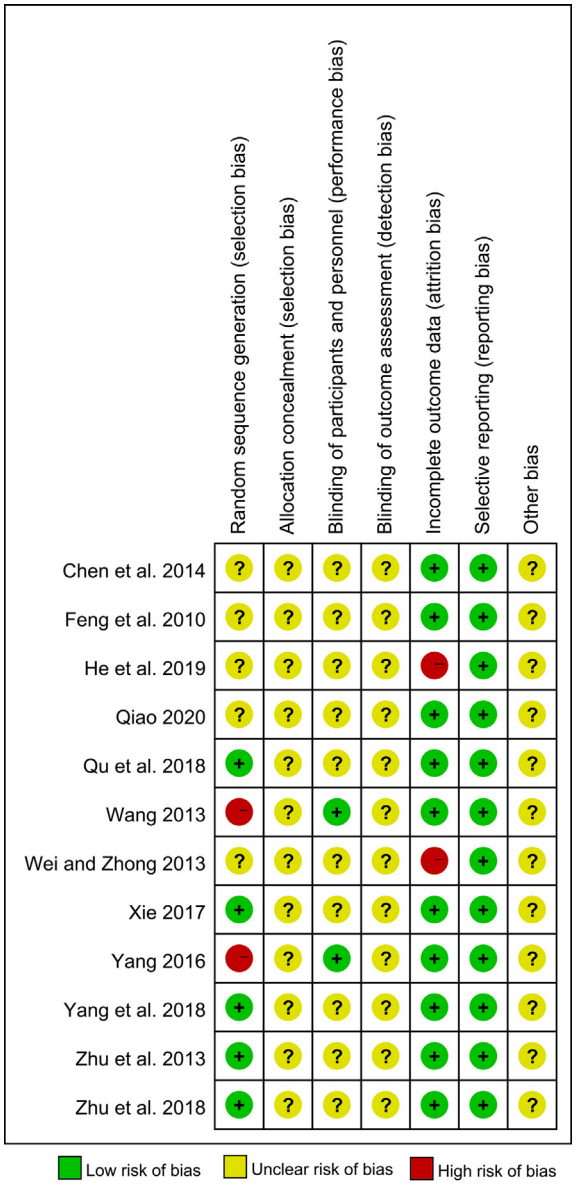

Fig. 2. Quality assessments of included studies.

3.4. Primary outcomes

3.4.1. Complete remission rate

Eight studies (Feng et al., 2010; Wei & Zhong, 2013; Zhu et al., 2013; Chen et al., 2014; Xie, 2017; Zhu et al., 2018; Qu et al., 2018; He et al., 2019) reported the complete remission rate. There was no significant heterogeneity (P = 0.62, I^2 = 0%) found, and a fixed effect model was utilized. Meta-analysis showed no significant difference in the complete remission rate after treatment between the RIF group and the ATO group (*RR* = 1.00, 95%*CI* [0.96, 1.05] P = 0.88; Fig. 3A).

3.4.2. CR time

Four studies (Wei & Zhong, 2013; Chen et al., 2014; Xie, 2017; He et al., 2019) reported this outcome. Heterogeneity tests showed that there was large heterogeneity in three studies (P = 0.002, I^2 = 80%), and the random effects model was utilized to analyze the data. The Meta-analysis showed no significant difference between the RIF group and the ATO group in reaching CR time (*RR* = − 0.84, 95%*CI* [−4.15, 2.48] P = 0.62; Fig. 3B).

3.4.3. 2-year DFS

Two studies (Zhu et al., 2013; Zhu et al., 2018) reported the patient's 2-year DFS rate. There was no significant heterogeneity (P = 0.99, I^2 = 0%) found, and a fixed effect model was used. The Meta-analysis showed no significant difference in 2-year DFS between the two groups (*RR* = 1.03, 95%*CI* [0.98, 1.07] P = 0.21; Fig. 3C).

3.4.4. Mortality

Eight studies (Feng et al., 2010; Wei & Zhong, 2013; Zhu et al., 2013; Chen et al., 2014; Xie, 2017; Zhu et al., 2018; He et al., 2019; Qiao, 2020) reported patients' mortality during treatment. There was no significant heterogeneity among the six studies (P = 0.51, I^2 = 0%), and a fixed effect model was used. The Meta-analysis showed no significant difference in mortality between the RIF group and the ATO group (*RR* = 0.81, 95%*CI* [0.38,1.75] P = 0.60; Fig. 3D).

3.4.5. Relapse rate

Five studies (Zhu et al., 2013; Chen et al., 2014; Zhu et al., 2018; Qu et al., 2018; Qiao, 2020) reported relapse rates. There was no significant heterogeneity (P = 0.90, I^2 = 0%) found, and a fixed effect model was used. The Meta-analysis showed no significant difference in the relapse rate between the RIF group and the ATO group (*RR* = 1.15, 95% *CI* [0.41, 3.24] P = 0.79; Fig. 3E).

3.5. Secondary outcomes

3.5.1. Peripheral blood (WBC, PLT)

Four studies (Feng et al., 2010; Wang, 2013; Yang, 2016; Xie, 2017) reported leukocyte levels, two of the (Wang, 2013; Yang, 2016) studies counted levels of white blood cells after maintenance treatment, the other two (Feng et al., 2010; Xie, 2017) studies counted the range of white blood cells in patients with first-onset after the treatment. Three studies (Feng et al., 2010; Wang, 2013; Yang, 2016) reported platelet levels. The heterogeneity analysis showed that the white blood cells (WBC) and platelets (PLT) indicators were not significantly heterogeneous (P = 0.99, I^2 = 0%;

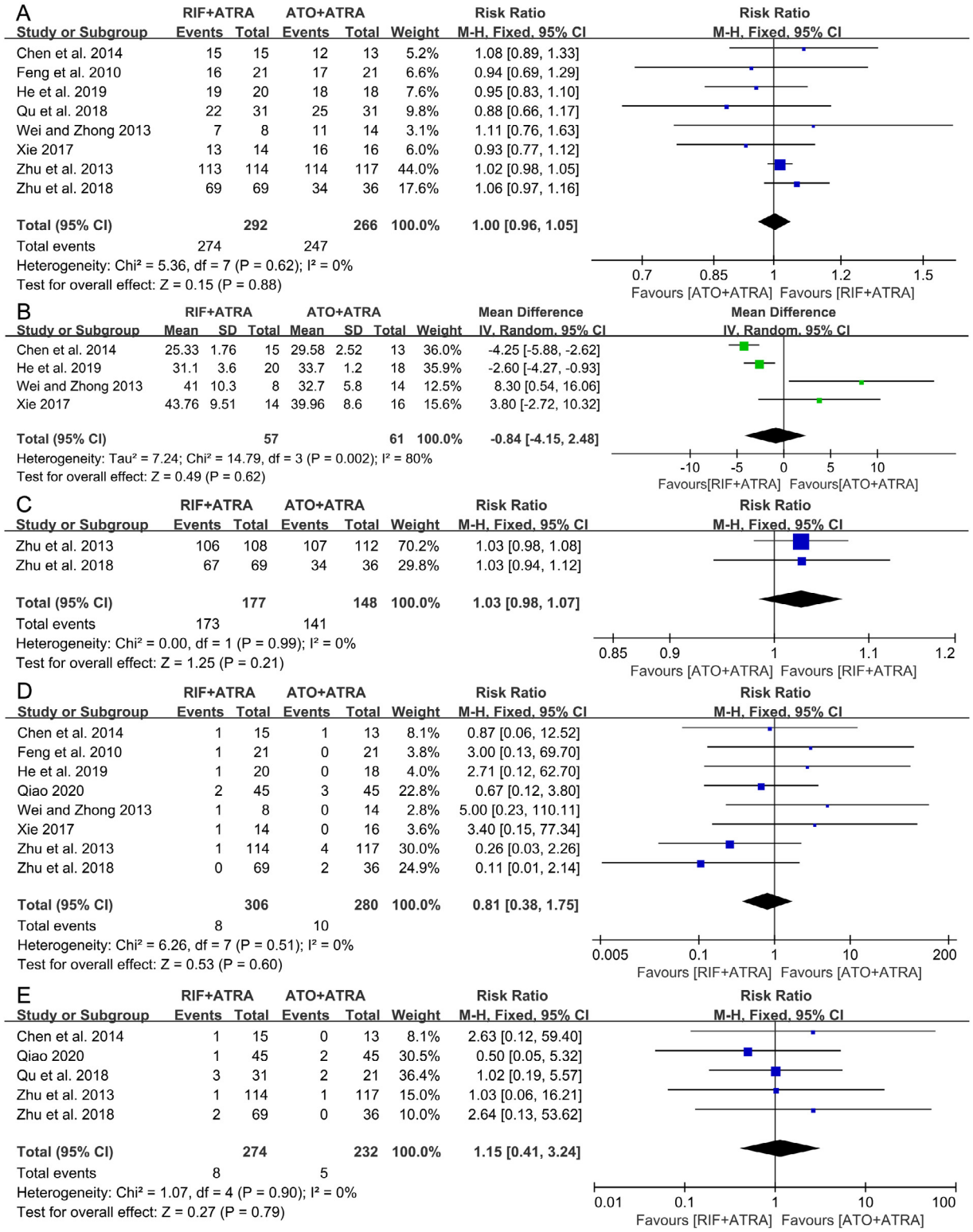

Fig. 3. Forest plot of the primary outcomes between RIF group and ATO group: Complete remission rate (A), CR time (B), 2-year DFS (C), mortality (D), relapse rate (E). ◆pooled RR,-■- RR and 95% *CI*.

P = 0.91, I^2 = 0%), and a fixed effect model was used. The Meta-analysis showed no significant difference in the blood tests for the two treatment regimens (*RR* = -0.19, 95% *CI* [−0.68, 0.30] P = 0.44); *RR* = 4.49, 95%*CI* [−2.43, 11.41] P = 0.20; Fig. 4A and B).

3.5.2. Biochemical indicators (ALT, AST, UA, UREA)

Three studies (Wang, 2013; Yang, 2016; Qu et al., 2018) measured alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), and urea levels after administration. Hetero-

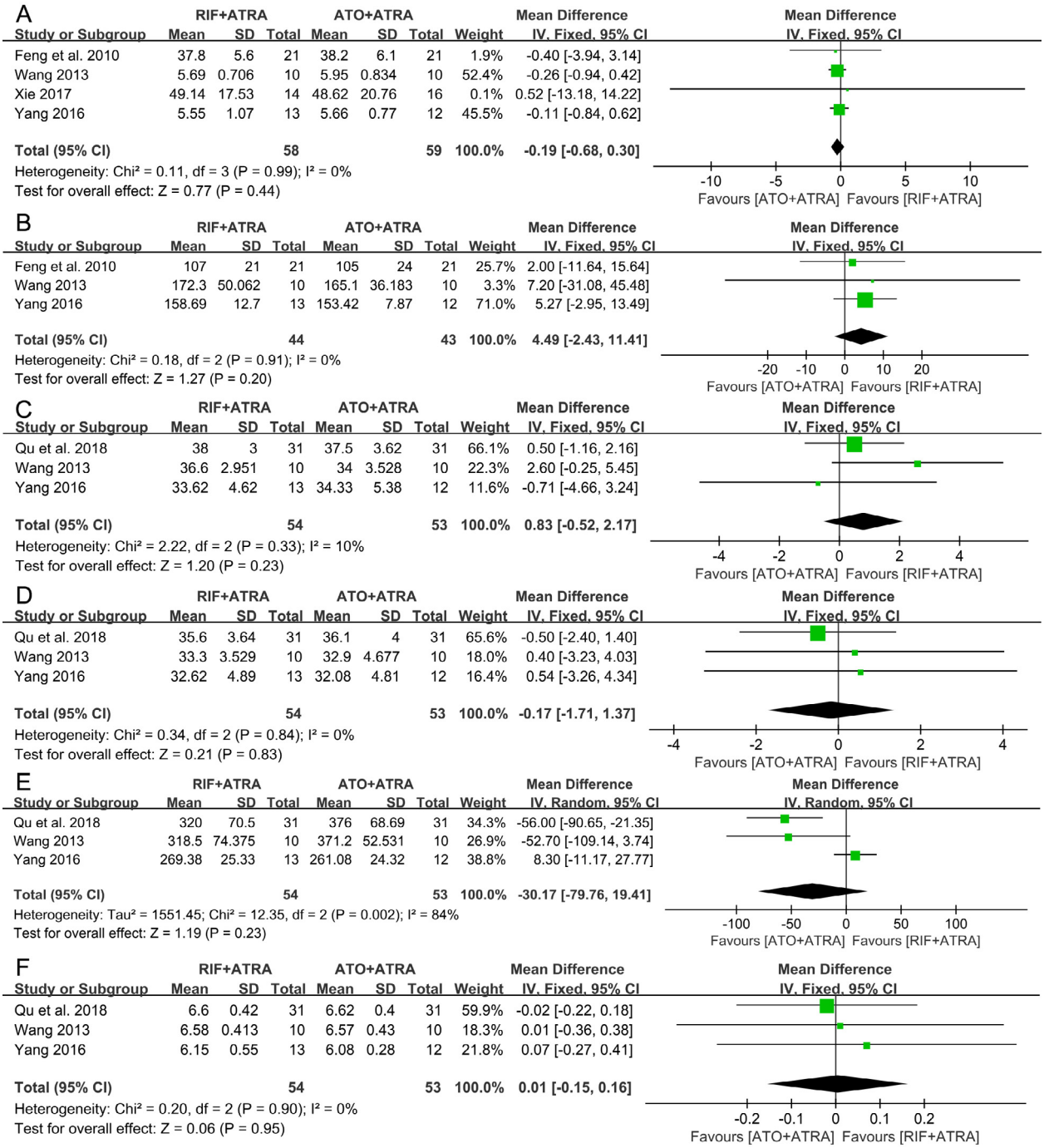

Fig. 4. Forest plot of peripheral blood (A, WBC; B PLT) and biochemical indicators (C, ALT; D, AST; E, UA; F, urea) of secondary outcomes between RIF group and ATO group,. ◆ pooled RR, -■- RR and 95% *CI*.

geneity analysis showed that ALT, AST and urea did not have significant heterogeneity (P = 0.33, I^2 = 10%; P = 0.84, I^2 = 0%; P = 0.90, I^2 = 0%, respectively), and a fixed effect model was used. UA was found to be heterogeneous (P = 0.002, I^2 = 84%), and a random effects model was used. The Meta-analysis showed no significant differences in the effect of the two treatment regimens on the biochemical indicators of the patients (*RR* = 0.83, 95% *CI* [−0.52, 2.17] P = 0.23; *RR* = − 0.17, 95% *CI* [−1.71, 1.37] P = 0.83; *RR* = − 30.17, 95% *CI* [−79.76, 19.41] P = 0.23; *RR* = 0.01, 95% *CI* [−0.15, 0.16] P = 0.95; Fig. 4 C–F).

3.5.3. Adverse events

In the RIF group and the ATO group, adverse reactions after taking the drug mainly included nausea, vomiting, diarrhea, liver dysfunction, and cardiac abnormalities. Because the statistical standards of each of the included studies were different, this study mainly analyzed six adverse reactions reported in the literatures. According to the statistics, five studies (Feng et al., 2010; Chen et al., 2014; Xie, 2017; Yang et al., 2018; Zhu et al., 2018) reported liver dysfunction; Four studies (Xie, 2017; Yang et al., 2018; Zhu et al., 2018; Qu et al., 2018) reported cardiac abnormalities; Four

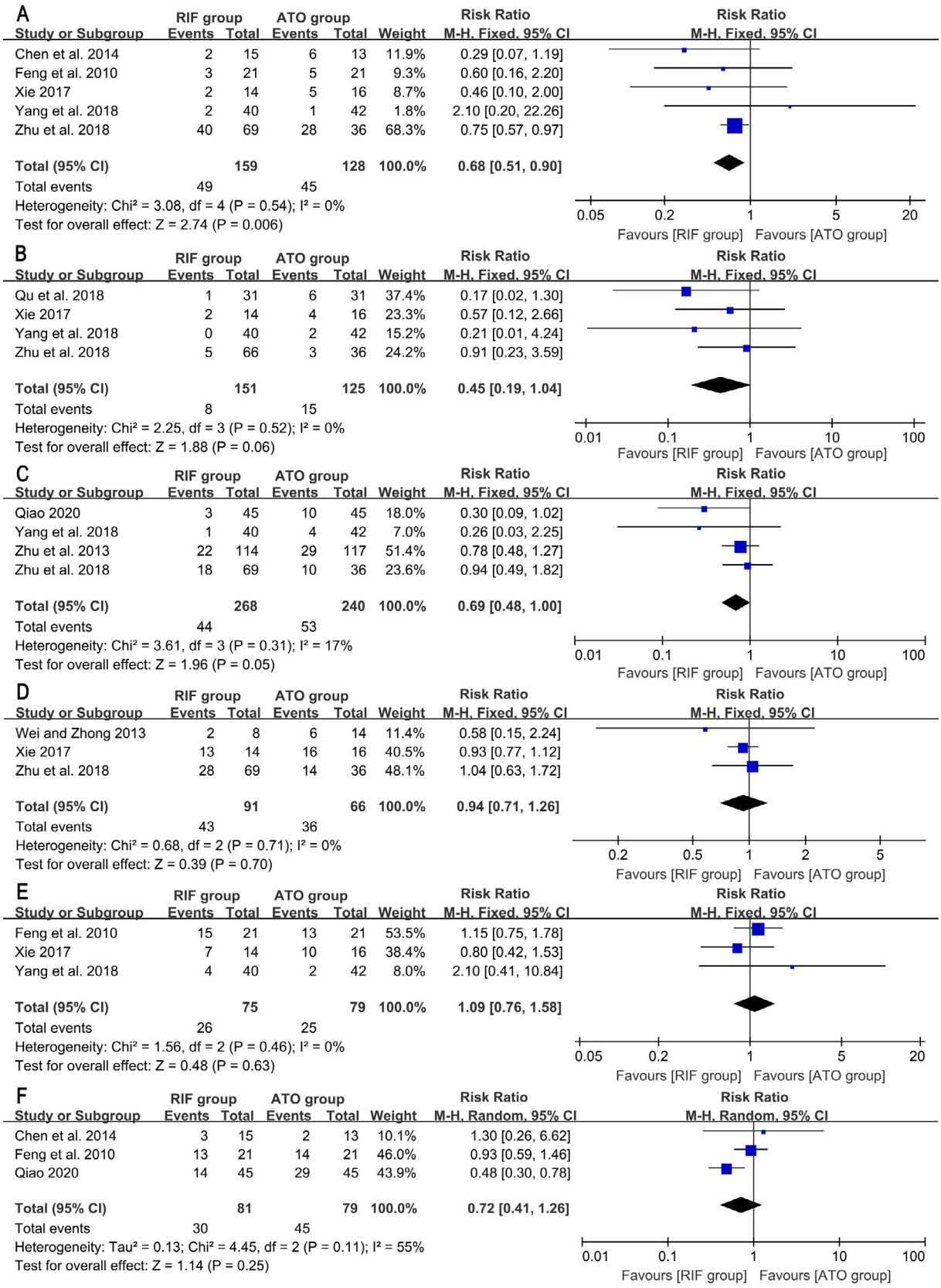

Fig. 5. Forest plot of adverse reactions of secondary outcomes between RIF group and ATO group. liver dysfunction (A), cardiac abnormalities (B), differentiation syndrome (C), hyperleukocytosis (D), coagulation abnormalities (E), and gastro-intestinal response (F). ◆ pooled RR, -■- RR and 95% CI.

studies (Zhu et al., 2013; Yang et al., 2018; Zhu et al., 2018; Qiao, 2020) reported the occurrence of differentiation syndrome; Three studies (Wei & Zhong, 2013; Xie, 2017; Zhu et al., 2018) reported hyperleukocytosis; Three studies (Feng et al., 2010; Xie, 2017; Yang et al., 2018) reported coagulation abnormalities, and three studies (Feng et al., 2010; Chen et al., 2014; Qiao, 2020) reported gastro-intestinal response. Heterogeneity analysis showed that the first five adverse reactions indicators were not significant heterogeneous (P = 0.54, I^2 = 0%; P = 0.52, I^2 = 0%; P = 0.31, I^2 = 17%; P = 0.71, I^2 = 0%; P = 0.46, I^2 = 0%, respectively), and a fixed effect model was used. The gastro-intestinal response was found to have significant heterogeneous (P = 0.11, I^2 = 55%), and a random effects model was used. The Meta-analysis showed that liver dysfunction was significantly reduced in the RIF group compared with the ATO group (*RR* = 0.68, 95% *CI* [0.51, 0.90] P = 0.006; Fig. 5A). In addition, compared with the ATO group, there were no significant differences in the incidence of adverse reactions, such as cardiac abnormalities, differentiation syndrome, hyperleukocytosis, coagulation abnormalities, and gastro-intestinal response in the RIF group (*RR* = 0.45, 95% *CI* [0.19, 1.04] P = 0.06; *RR* = 0.69, 95% *CI* [0.48, 1.00] P = 0.05; *RR* = 0.94, 95% *CI* [0.71, 1.26] P = 0.70]; *RR* = 1.09, 95% *CI* [0.76, 1.58] P = 0.63; *RR* = 0.72, 95% *CI* [0.41, 1.26] P = 0.25; Fig. 5 B–F).

3.5.4. Treatment cost

Three studies (Wang, 2013; Yang, 2016; He et al., 2019) counted expenditure on patients after treatment. Descriptive analyses were performed because the data gap was too large. According to statistics from Xiu-ping Wang (Wang, 2013), the cost of the experimental group in each of the five maintenance treatment cycles performed by the patients was significantly lower than that of the control group (P < 0.05). From the statistical results of the study by Shi-yu Yang (Yang, 2016), it can be seen that the total cost of patients in the experimental group during the whole maintenance period (¥15870.23 ± 534.995) was significantly lower than that of the control group (¥49484.42 ± 1902.95) (P < 0.05). In addition, He et al. (He et al., 2019) showed that the cost of the two groups of patients during the induction treatment period was not significantly different (P > 0.05), but the total cost of the seven consolidation treatments was significantly lower in the experimental group (¥76331.1 ± 21.1) than in the control group (¥114153.2 ± 45.5) (P < 0.05). In general, there was no difference in treatment between the two groups except for the type of arsenic, it can be seen that oral RIF combined with ATRA-based treatment has obvious economic advantages.

3.6. Publication bias

This study used a funnel chart to evaluate the publication bias of the included studies, and the results showed that the included studies were not symmetrical (Fig. 6A), combined with the analysis results of the Egger and Begg tests, there was no significant risk of bias in the included studies (Egger: P = 0.502; Begg (Fig. 6B): P = 0.711).

3.7. Sensitivity analysis

The above Meta-analysis results showed that the heterogeneity of most indicators was small. In order to detect hidden heterogeneity in the analysis results, a sensitivity analysis of the data under the random effects model and the fixed effect model was carried out. It can be seen from the statistical results (Table 2) that the indexes with smaller heterogeneity used the effective combination of the two effect models and that the 95%*CI* did not change much for indicators with lower heterogeneity. However, for the three indicators with larger heterogeneity (CR Time, UA and gastro-intestinal response), the results of applying the two models were quite different. This phenomenon indicated that the heterogeneity test results of all indicators in this system were stable.

3.8. Network pharmacology results of RIF in treatment of APL

According to the screening conditions, a total of 59 potential active ingredients related to RIF were retrieved in the TCMSP and Batman databases, and a total of 217 potential targets were obtained. In addition, 1309 APL-related targets were obtained through the OMIM database and the Drug Bank database. Then, we conducted network associations for "drugs", "drug targets" and "disease targets" (Fig. 7), and found that a total of 46 targets were co-regulatory targets for drugs and diseases. In order to further search targets with higher correlation, we analyzed and screened the topological parameters of 46 targets, and take the median of the three topological parameters as the screening criteria. Ultimately, a total of 19 targets with most valuable for research were obtained (Table 3). Subsequently, in order to further clarify the pharmacodynamic mechanism of RIF, we conducted a Reactome enrichment analysis for the above 19 core targets (Table 3). Subsequently, Reactome enrichment analysis results for the above 19 core targets indicate that RIF may play a therapeutic effect on APL by regulating four biological mechanisms (TP53 Regulates Transcription of Cell Cycle Genes, nuclear receptor transcription pathway, Regulation of PTEN gene transcription, and Intrinsic Pathway for Apoptosis) (Fig. 8).

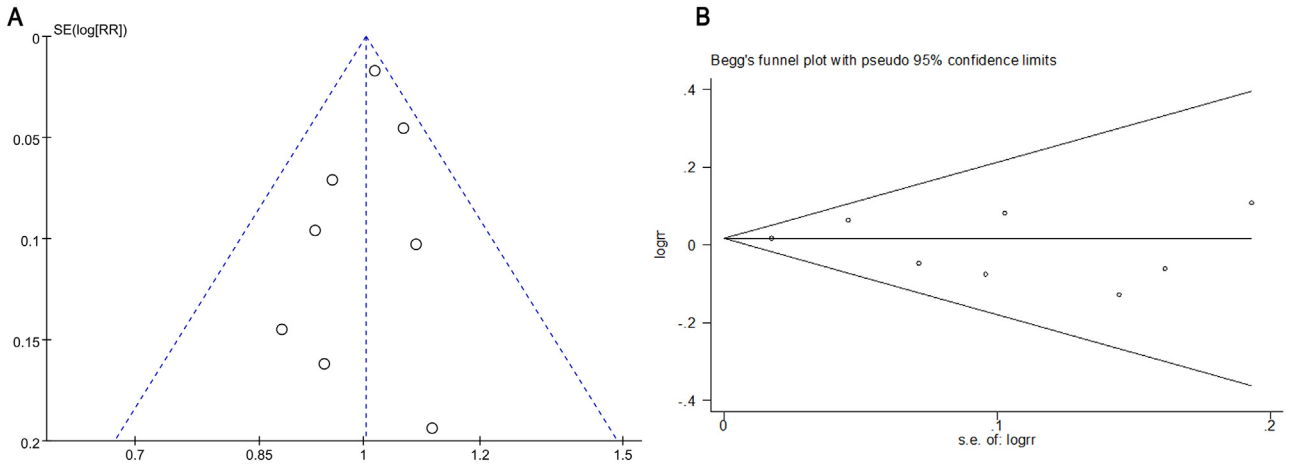

Fig. 6. Funnel diagram of complete remission rate (A) and Begg's funnel plot (B) between RIF group and ATO group.

Table 2
Effect size and 95%CI under random effects model and fixed effect model of literatures included in this study.

Outcomes	Fixed model	Random model
CRR	*RR* = 1.00, 95% *CI*: 0.96–1.05	*RR* = 1.02, 95% *CI*:0.99–1.05
CR time	*MD* = − 2.97, 95% *CI*:-4.11–1.83	*MD* = − 0.84, 95% *CI*:-4.15–2.48
2-year disease-free survival	*RR* = 1.03, 95% *CI*:0.98–1.07	*RR* = 1.03, 95% *CI*:0.99–1.07
mortality	*RR* = 0.81, 95% *CI*:0.38–1.75	*RR* = 0.86, 95% *CI*:0.34–2.14
remission rate	*RR* = 1.15, 95% *CI*:0.41–3.24	*RR* = 1.11, 95% *CI*:0.38–3.25
WBC	*MD* = − 0.19, 95% *CI*:-0.68–0.30	*MD* = − 0.19, 95% *CI*:-0.68–0.30
PLT	*MD* = 4.49, 95% *CI*:-2.43–11.41	*MD* = 4.49, 95% *CI*:-2.43–11.41
ALT	*MD* = 0.83, 95% *CI*:-0.52–2.17	*MD* = 0.85, 95% *CI*:-0.63–2.33
AST	*MD* = − 0.17, 95% *CI*:-1.71–1.37	*MD* = − 0.17, 95% *CI*:-1.71–1.37
UA	*MD* = − 10.91, 95% *CI*:-27.16–5.35	*MD* = − 30.17, 95% *CI*:-79.76–19.41
UREA	*MD* = 0.01, 95% *CI*:-0.15–0.16	*MD* = 0.01, 95% *CI*:-0.15–0.16
liver dysfunction	*RR* = 0.68, 95% *CI*:0.51–0.90	*RR* = 0.72, 95% *CI*:0.56–0.92
cardiac abnormalities	*RR* = 0.45, 95% *CI*:0.19–1.04	*RR* = 0.51, 95% *CI*:0.21–1.22
differentiation syndrome	*RR* = 0.69, 95% *CI*:0.48–1.00	*RR* = 0.71, 95% *CI*:0.46–1.10
hyperleukocytosis	*RR* = 0.94, 95% *CI*:0.71–1.26	*RR* = 0.93, 95% *CI*:0.78–1.11
coagulation abnormalities	*RR* = 1.09, 95% *CI*:0.76–1.58	*RR* = 1.06, 95% *CI*:0.75–1.51
gastrointestinal reactions	RR = 0.66, 95% *CI*:0.47–0.92	RR = 0.72, 95% *CI*:0.41–1.26

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CR = complete remission; CRR = complete remission rate; DFS = disease-free survival; MD = mean differences; PLT = platelets; RR = relative risk; CI = confidence interval; UA = uric acid; WBC = white blood cells.

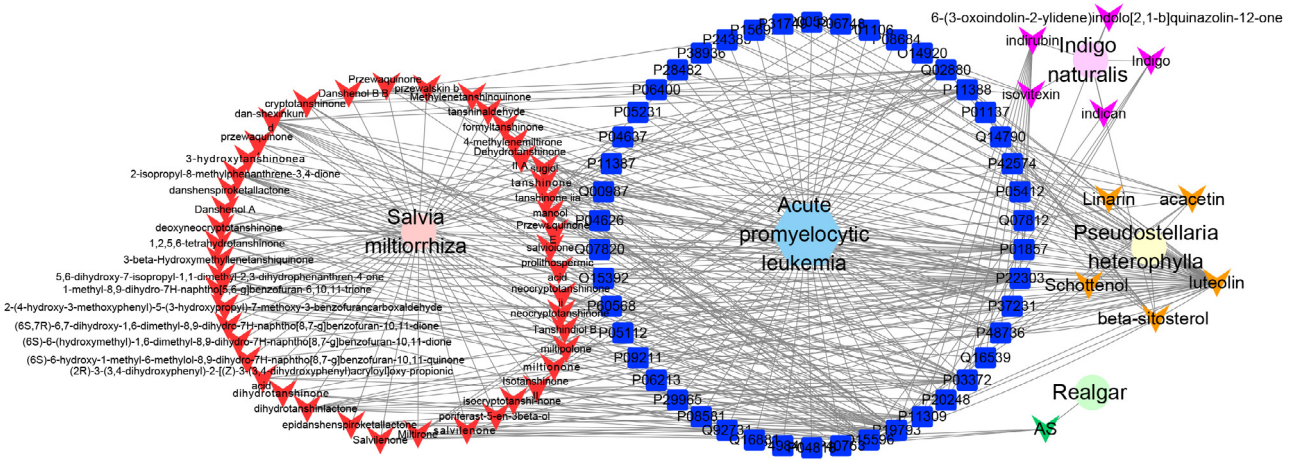

Fig. 7. "Drug-drug target-disease target" network of RIF treatment of APL.

Table 3
Core targets of RIF in treatment of APL.

Gene names	Closeness	Degree	Betweenness	Core targets
ACHE	0.47767857	26	0.10206803	P22303
NCOA2	0.46929825	25	0.09415366	Q15596
RXRA	0.47767857	26	0.06672945	P19793
ESR1	0.4612069	22	0.06310412	P03372
TOP2A	0.44214876	17	0.0409161	P11388
PIK3CG	0.43145161	14	0.03344979	P48736
TOP2B	0.43852459	15	0.03198421	Q02880
IGHG1	0.43852459	16	0.02394495	P01857
PPARG	0.428	13	0.02069588	P37231
PIM1	0.428	13	0.01954974	P11309
CCNA2	0.41796875	10	0.01330194	P20248
ESR2	0.39925373	5	0.00512193	Q92731
CASP3	0.40530303	6	0.00430563	P42574
JUN	0.40225564	5	0.00265452	P05412
CDKN1A	0.39925373	5	0.00256599	P38936
TP53	0.39925373	5	0.00256599	P04637
MAPK14	0.3962963	3	0.0022626	Q16539
CASP8	0.3962963	3	0.00171679	Q14790
BAX	0.3962963	3	0.00171679	Q07812

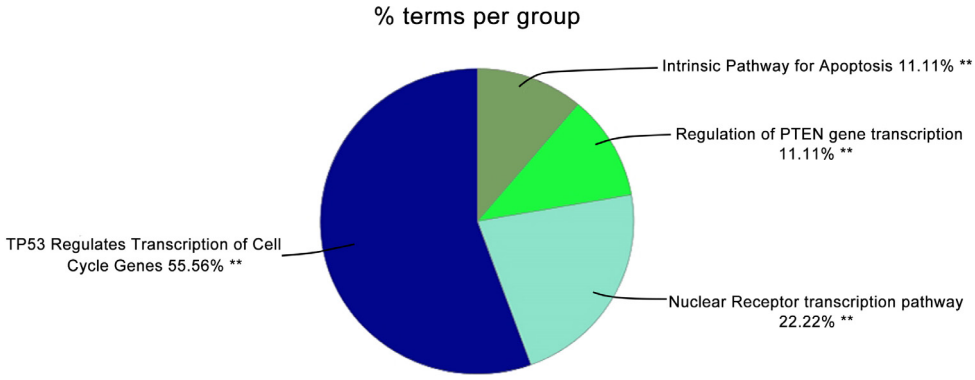

Fig. 8. Results of Reactome pathway analysis.

4. Discussion

This study used an evidence-based approach to conduct a Meta-analysis of the safety and efficacy of oral RIF combined with ATRA for the treatment of APL. According to the results of the Meta-analysis, differences in complete remission rate (P = 0.88), CR time (P = 0.62), 2-year DFS (P = 0.21), mortality (P = 0.60), relapse rate (P = 0.79), WBC (P = 0.44), PLT (P = 0.20), ALT (P = 0.23), AST (P = 0.83), UA (P = 0.23) and UREA (P = 0.95) between the RIF group and the ATO group were not obvious. However, the incidence of abnormal liver function (P = 0.006) in the RIF group were significantly lower than that in the ATO group, but there was no significant difference in the incidence of cardiac abnormalities (P = 0.06), differentiation syndrome (P = 0.05), hyperleukocytosis (P = 0.70), coagulation abnormalities (P = 0.63) and gastro-intestinal response (P = 0.05), which suggests that oral RIF combined with ATRA is safer. Descriptive analysis showed that compared with the ATO group, patients in the RIF group had significantly lower treatment costs during the maintenance treatment period. It can be seen that there is no significant difference in efficacy between the two regimens, and oral RIF + ATRA is safer to treat APL and can significantly reduce the economic burden on patients.

From the perspective of the chain of evidence, RIF-based oral arsenic regimen was a more recommended therapy, and from the perspective of systems biology, pure Chinese medicinal formulae RIF also had its unique advantages for the treatment of APL. In recent years, as the pathological research of APL had gradually deepened, studies have shown that the molecular mechanism of APL was that PML-RARα causes cell differentiation to be blocked, apoptosis was inhibited and cell self-renewal was abnormal, and so on (Kumar & Tchounwou, 2015).

The results of network pharmacology analysis in this study showed that the targets and pathways of RIF in the treatment of APL could play a therapeutic role in the above pathological mechanisms. The results of the Reactome enrichment analysis found that RIF mainly regulates the apoptosis pathway by acting on TP53 gene and RIF also regulated nuclear receptor transcription pathways to inhibit APL cell proliferation, induce differentiation and apoptosis (Pan & Chen, 2020; Swaney et al., 2016). Therefore, whether from the perspective of evidence-based medicine or systems biology, RIF has its unique advantages.

Anyway, clinical application of the oral RIF + ATRA regimen for the treatment of APL has the advantages of being convenient to administer, allowing patients to avoid intravenous injection, and reducing the incidence of nosocomial infections and the use of chemotherapy drugs, advantages that are extremely rare in the field of cancer treatment. According to the study by Zhu et al., using only RIF combined with ATRA to treat APL, it will be possible to achieve a new treatment mode for APL without chemotherapy, without infusions, and with only two oral drugs (Zhu & Huang, 2014). Furthermore, this study combines evidence-based medicine research methods with systematic pharmacology research methods. Meta-analysis results showed that RIF have good efficacy and safety as an auxiliary drug for the treatment of APL, and network pharmacology studies are used to initially reveal its potential mechanism of action, laying the foundation for the follow-up clinical application and experimental research of RIF.

However, this Meta-analysis was limited by the number and quality of the randomized controlled trials, reducing the strength of the study. In addition, it is very regrettable that the statistical criteria for the literature outcome indicators included in this paper are different, resulting in some typical outcome indicators (such as treatment costs) not included in the Meta-analysis. In the future, we need to conduct large-scale and high-quality RCTs to further confirm the efficacy, safety and economic benefits of oral RIF combined with ATRA for the treatment of APL.

5. Conclusion

This study found that RIF mainly regulates cell apoptosis and inhibits cell proliferation through regulating multiple molecular mechanisms, such as TP53 regulates transcription of cell cycle genes, nuclear receptor transcription pathway and other pathways, then improve the disease severity of APL patients. And for the treatment of APL, the oral regimen (RIF combined with ATRA) is equivalent to the injection regimen (ATO combined with ATRA). In addition, in terms of safety and economic benefits, oral regimen is superior to injection regimen. Nevertheless, the follow-up still needs to further include more high-quality RCTs to verify the above conclusions.

Authors' Contributions

Wenjun Zou and Qiaozhi Yin conceived and designed the study; Qianqian Huang, Tao Wang and Yan Xiong collected the data; Qian qian Huang, Tao Wang performed the analysis and prepared the manuscript; Wenjun Zou, Liping Qu and Qiaozhi Yin made amendments to the manuscript. All authors read and approved the final version of the manuscript.

Statement

This study is a Meta-analysis and systematic review, based on the published clinical literature data. So, the ethical review was not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Adams, J., & Nassiri, M. (2015). Acute promyelocytic leukemia: A review and discussion of variant translocations. *Archives of Pathology & Laboratory Medicine, 139*(10), 1308–1313.

Bernard, J., Weil, M., Boiron, M., Jacquillat, C., & Gemon, M.F. (1973). Acute promyelocytic leukemia: Results of treatment by Daunorubicin. *Blood*, 41(4), 489-496.

Blackburn, L., Bauchmire, N., Bender, S., Tomlinson-Pinkham, K., Roberts, S., & Rosan, S. (2016). Impact of an alert system on quality indicators in patients with acute promyelocytic leukemia. *Clinical Journal of Oncology Nursing, 20*(5), 523–527.

Braess, J. (2016). *Acute myeloid leukemia. Deutsche medizinische Wochenschrift, 141*(24), 1748–1751.

Chen, J. M., Cai, N., & Chen, J. L. (2014). Clinical observation on treatment of acute promyelocytic leukemia with combination of traditional Chinese medicine and western medicine. *Journal of Practical Traditional Chinese Medicine, 6*, 524–525.

Chen, Z., & Chen, S. J. (2017). Poisoning the Devil. *Cell, 168*(4), 556–560.

Feng, Y. H., Huang, M., & Xu, L. Y. (2010). Comparison of the efficacy of all-*trans* retinoic acid combined with Baixuekang and arsenic trioxide in the treatment of acute promyelocytic leukemia. *Journal of Bengbu Medical College, 35*(7), 709–711.

Giri, S., Pathak, R., Aryal, M. R., Karmacharya, P., & Bhatt, V. R. (2017). Second primary malignancy in acute promyelocytic leukemia: A Surveillance, Epidemiology and End Results database study. *Future Oncology, 13*(17), 1455–1457.

He, Q.L., Liu, Y., Wei, J., Zou, X.L., Ma, C.H.R., & Cao, H. (2019). Clinical study on the clinical efficacy and economy of retinoic acid combined with Realgar-Indigo Naturalis Formula Tablets in the treatment of non-high risk acute promyelocytic leukemia. *Journal of Chengdu Medical College*, 14(5), 611-615 +620.

Higgins, J. P. T., & Green, S. (2011). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. *The Cochrane Collaboration*.. Accessed 1 October 2018 http://www.cochrane-handbook.org.

Hu, X. M., Liu, F., & Ma, R. (2010). Application and assessment of Chinese arsenic drugs in treating malignant hematopathy in China. *Chinese Journal of Integrative Medicine, 16*(4), 368–377.

Jiang, H., Liang, G. W., Huang, X. J., Jiang, Q., Han, S., Shi, L. W., & Zhu, H. H. (2015). Reduced medical costs and hospital days when using oral arsenic plus ATRA as the first-line treatment of acute promyelocytic leukemia. *Leukemia Research, 39*(12), 1319–1324.

Kumana, C. R., Au, W. Y., Lee, N. S., Kou, M., Mak, R. W., Lam, C. W., & Kwong, Y. L. (2002). Systemic availability of arsenic from oral arsenic-trioxide used to treat patients with hematological malignancies. *European Journal of Clinical Pharmacology, 58*(8), 521–526.

Kumana, C.R., & Kuang, Y. (2006). Formulations of oral compositions including arsenic trioxide and methods of use thereof: China, CN200380105498.8. 2006-01-18.

Kumar, S., & Tchounwou, P. B. (2015). Molecular mechanisms of cisplatin cytotoxicity in acute promyelocytic leukemia cells. *Oncotarget, 6*(38), 40734–40746.

NCCN. Guidelines for Patients: Acute Myeloid Leukemia in 2020. (2020). https://www.nccn.org/patients/guidelines/content/PDF/aml-patient.pdf. Accessed 12 June 2020.

Pan, P., & Chen, X. (2020). Nuclear receptors as potential therapeutic targets for myeloid leukemia. *Cells, 9*, 1921.

Qiao, B. (2020). Efficacy and safety of Compound Huangdai Tablets combined with retinoic acid in treatment of patients with acute promyelocytic leukemia in maintenance phase. *Journal of Clinical Medicine in Practice*, 24(12), 114-116+124.

Qu, M., Liang, H., Zhang, T., & Cui, X. H. (2018). Therapeutic effect of compound Huangdai Tablet on acute promyelocytic leukemia. *Chinese Archives of Traditional Chinese Medicine, 36*(7), 1683–1686.

Rao, Y., Li, R. H., & Zhang, D. Q. (2013). Poisoning as medicine: Discovery of the effect of arsenic trioxide on acute promyelocytic leukemia. *Scientia Sinica (Vitae), 43*(8), 700–707.

Ribeiro, R. C., & Rego, E. (2006). Management of APL in developing countries: Epidemiology, challenges and opportunities for international collaboration. *Hematology. American Society of Hematology. Education Program, 2006*(1), 162–168.

Swaney, E. M., Chattopadhyay, A., Abecassis, I., Rush, E. A., & Redner, R. L. (2016). The leukemic oncoprotein NPM1-RARA inhibits TP53 activity. *Leukemia & Lymphoma, 57*(8), 1933–1937.

The hematology branch of Chinese Medical Association., & Chinese Medical Doctor Association of Hematologists (2018). Guidelines for the diagnosis and treatment of acute promyelocytic leukemia in China (version 2018). *Chinese Journal of Hematology, 39*(3), 179–183.

Wang, L., Zhou, G. B., Liu, P., Song, J. H., Liang, Y., Yan, X. J., ... Chen, Z. (2008). Dissection of mechanisms of Chinese medicinal formula *Realgar-Indigo Naturalis* as an effective treatment for promyelocytic leukemia. *Proceedings of the National Academy of Sciences of the United States of America, 105*(12), 4826–4831.

Wang, X. P. (2013). *Clinical control observation of compound Huangdai Tablet in the treatment of acute promyelocytic leukemia*. Guangzhou: Guangzhou University of Chinese Medicine.

Wang, Z. Y., & Chen, Z. (2008). Acute promyelocytic leukemia: From highly fatal to highly curable. *Blood, 111*(5), 2505–2515.

Wei, H. Q., & Zhong, W. Y. (2013). Efficacy of compound Huangdai Tablets combined with all-trans retinoic acid in the treatment of newly diagnosed acute promyelocytic leukemia. *Chinese Journal of Medicinal Guide, 11*, 1864–1865.

Xie, S. J. (2017). *Clinical study on the treatment of acute promyelocytic leukemia with compound Huangqdai tablets combined with retinoic acid*. Guangzhou: Guangzhou University of Chinese Medicine.

Xu, H. H., Hao, F. R., Wang, M. X., Ren, S. J., Li, M., Tan, H. L., ... Ma, Z. C. (2017). Influences of *Realgar-Indigo Naturalis*, A traditional Chinese medicine Formula, on the main CYP450 activities in rats using a Cocktail method. *Evidence-Based Complementary and Alternative Medicine, 2017*, 1–9.

Yang, M. H., Wan, W. Q., Luo, J. S., Zheng, M. C., Huang, K., Yang, L. H., ... Luo, X. Q. (2018). Multicenter randomized trial of arsenic trioxide and *Realgar-Indigo Naturalis* formula in pediatric patients with acute promyelocytic leukemia: Interim results of the SCCLG-APL clinical study. *American Journal of Hematology, 93*(12), 1467–1473.

Yang, S. H. Y. (2016). *Clinical observation of oral arsenic in the treatment of acute promyelocytic leukemia*. Guangzhou: Guangzhou University of Chinese Medicine.

Zhang, M., Guo, L., Lin, L. F., Qu, C. H., Yin, X. B., Luo, S. L., ... Ni, J. (2018). Absorption characteristics of combination medication of *Realgar* and *Indigo Naturalis*: *In vitro* transport across MDCK-MDR1 cells and *in vivo* pharmacokinetics in mice after oral administration. *Evidence-Based Complementary and Alternative Medicine, 2018*, 1–10.

Zhu, H. H., & Huang, X. J. (2014). Oral arsenic and retinoic acid for non-high-risk acute promyelocytic leukemia. *New England Journal of Medicine, 371*(23), 2239–2241.

Zhu, H. H., Wu, D. P., Du, X., Zhang, X., Liu, L., Ma, J., ... Huang, X. J. (2018). Oral arsenic plus retinoic acid versus intravenous arsenic plus retinoic acid for non-high-risk acute promyelocytic leukemia: A non-inferiority, randomized phase 3 trial. *Lancet Oncology, 19*(7), 871–879.

Zhu, H. H., Wu, D. P., Jin, J., Li, J. Y., Ma, J., Wang, J. X., ... Huang, X. J. (2013). Oral Tetra-arsenic tetra-sulfide formula versus intravenous arsenic trioxide as first-line treatment of acute promyelocytic leukemia: A multicenter randomized controlled trial. *Journal of Clinical Oncology, 31*(33), 4215–4221.