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Research

Treatment-free remission after discontinuation of tyrosine kinase inhibitors in patients with chronic myeloid leukemia in the chronic phase: a systematic review and meta-analysis

Zhenxiang Zheng[1] · Hao Tang[1] · Xinxia Zhang[1] · Liling Zheng[2] · Zhao Yin[2] · Jie Zhou[3] · Yangmin Zhu[2]

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Abstract

Background Treatment-free remission (TFR) is a new long-term goal for treating selected patients with chronic myeloid leukemia in the chronic phase (CML-CP). Still, the appropriate group in which TFR can be attempted and the factors influencing it have not yet been identified. This meta-analysis aimed to explore TFR in CML-CP patients who achieved a deep molecular response (DMR) before Tyrosine kinase inhibitors (TKIs) discontinuation and to explore possible factors influencing TFR and the safety of discontinuation.

Methods We performed a systematic review and single-arm meta-analysis with a systematic search of published literature up to September 2023 in PubMed, Embase, Web of Science, Cochrane Library, and CNKI databases. The assessment was performed using the MINORS scale. Random-effects models were used to calculate outcome metrics, including overall mean TFR rates at 12 and 24 months and subgroup differences. Data synthesis and analysis were done by Stata17.0 software.

Results A total of 19 single-arm trials involving 2336 patients were included in this meta-analysis, with an overall mean TFR rate of 59% [95CI:0.56–0.63] at 12 months and 55% [95CI:0.52–0.59] at 24 months, and no CML-related deteriorations or deaths reported during the TFR period. Our subgroup analysis showed that better TFR was associated with prior interferon therapy (P = 0.003), and molecular response depth MR5.0 (P = 0.020).

Conclusion Our study demonstrated that prior interferon therapy and attainment of a molecular response depth of MR5.0 or greater were associated with higher TFR rates, with patients who attained MR5.0 or greater achieving a TFR rate of up to 62% in the second year after TKI discontinuation. Considering the high heterogeneity of the included trials, the above influences still require further validation and more detailed subgroup analysis in future discontinuation trials. *Systematic review registration:* https://www.crd.york.ac.uk/prospero/ (Registration No. CRD42023471334).

Keywords Treatment-free remission · Tyrosine kinase inhibitors · Chronic myeloid leukemia

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✉ Liling Zheng, 49695301@qq.com; ✉ Zhao Yin, zhaoyin@stu2014.jnu.edu.cn; ✉ Jie Zhou, 20336922@qq.com; ✉ Yangmin Zhu, zhuyangmin2005@163.com | [1]Jinan University, Guangzhou 510632, Guangdong, China. [2]Department of Hematology, Guangdong Second Provincial General Hospital, Jinan University, Xin Gang Zhong Road 466#, Haizhu Distict, Guangzhou 510317, Guangdong, China. [3]Deyang People's Hospital, Deyang 618199, Sichuan, China.

1 Introduction

Chronic myeloid leukemia (CML) is the result of the transformation of primitive hematopoietic cells by the breakpoint cluster region protein (BCR)/Abbelsen murine leukemia virus oncogene homologue (ABL) oncogene [1]. Since the advent of the first-generation BCR-ABL1 tyrosine kinase inhibitor (TKI), imatinib, it has been superior to interferon plus low-dose cytarabine as a first-line treatment for newly diagnosed CML-CP in terms of efficacy, tolerability, and the likelihood of progression to accelerated-phase or blast-crisis CML [2, 3]. Subsequent second-generation TKIs, demonstrated significantly higher and faster induction of complete cytogenetic responses and major molecular response rates. The prognosis for patients with CML has improved dramatically, more patients achieve a deep molecular response. [4–6]. Currently, first-line TKIs for the treatment of CML include imatinib, nilotinib, dasatinib, radotinib, and bosutinib et al. [7]. There are still some patients who are resistant to TKIs, mainly due to mutations in the structural domain of BCR-ABL kinase. Third-generation TKIs, such as ponatinib and asciminib, are used as second-line TKI therapy for patients previously resistant or intolerant to TKIs. [8, 9].

However, patients on long-term medication face complex chronic adverse effects and carry a heavy financial burden [5, 10, 11]. The STIM trial [12] demonstrated for the first time that TKI discontinuation was achievable in a subset of patients achieving CMR, and treatment-free remission became a new long-term goal for patients with CML-CP. A study by Aaron N Winn and his colleagues showed that attempts to discontinue TKI therapy could save more than $54 billion over the next 30 years [13].

Nevertheless, many questions remain unanswered, such as, which patients are the best group to try TFR, and which factors predict loss of major molecular response after discontinuation. The European Leukemia Expert Consensus recommends the duration of DMR before discontinuation to be at least 2–3 years [7], the Los Angeles CML Expert Consensus suggests it to be at least 2 years [14], whereas the Canadian trial suggests that this time should be extended to more than 6 years [15]. Since the first clinical trial of imatinib discontinuation, most of the multiple clinical trials in the last decade have demonstrated the feasibility and safety of discontinuation, Research conducted in 2020 has shown that it is safe to resume treatment with TKIs after unsuccessful attempts at TFR, as demonstrated by a meta-analysis [16]. Another meta-analysis from 2019 found that molecular relapse rates after discontinuation of TKIs were studied but with limited databases and trials [17]. Furthermore, a separate meta-analysis from the same year focused solely on the impact of study-level factors on second-year TFR rates [18]. There is still a lack of comprehensive and systematic analytic studies, and research on the long-term discontinuation of TKIs in CML patients in the post-TKI era still needs to be updated and improved.

Therefore, this systematic review will study the treatment-free remission after discontinuation of TKIs in patients with CML-CP through meta-analysis, explore the possible influencing factors of treatment-free remission as well as the safety, and further discuss the optimal patient population in which TFR can be attempted to provide evidence-based medical support for clinical guidelines.

2 Methods

This systematic review was conducted in accordance with Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines [19] and has been registered with PROSPERO (ID: CRD42023471334).

2.1 Search strategy

A systematic literature search was conducted utilizing five databases, PubMed, Embase, Web of Science, Cochrane Library, and CNKI. The time was limited from the time of library construction to September 2023, and the language was limited to English and Chinese. Literature was screened using endnote with the search formula: (Chronic myeloid leukemia AND CML) AND (imatinib OR nilotinib OR dasatinib OR bosutinib OR radotinib OR flumatinib OR tyrosine kinase inhibitor OR TKI) AND (stop OR withdrawal OR discontinue OR discontinuation OR treatment-free remission OR treatment free remission). (Detailed search formula in Supplement).

2.2 Selection criteria

2.2.1 Inclusion criteria

(1) adult patients with a diagnosis of CML-CP were included; (2) patients were treated with TKIs; (3) first attempt to discontinuation after achieving a deep molecular response (DMR); (4) prospective randomized clinical trials (RCTs), prospective non-RCTs; and (5) molecular relapse was defined as the loss of the major molecular response (MMR) or the loss of MR4.0;

2.2.2 Exclusion criteria

(1) Reviews or meta-analyses; (2) Case reports, Replies, or comments; (3) Lack of required TFR rate; (4) Pediatric patients; (5) Retrospective trials; (6) Basic experiments with cells or animals.

We extracted outcomes from the most complete and recent articles if the data from the same trial were published more than once. Two researchers (Tang and Zhang) independently screened the abstracts as well as the titles, and then read the full text of the relevant studies. Any disagreements were resolved by consensus or by a third reviewer (Zheng).

2.3 Outcome indicators

Primary outcome: Number of patients in treatment-free remission (TFR) and treatment-free remission rate at 12, 24 months after discontinuation;

Secondary outcome: cumulative adverse reaction rate, number of people experiencing CML progression, and CML-related deaths after discontinuation.

2.4 Data extraction

Two investigators (Tang AND Zhang) independently extracted the first author, time of publication, type of study, sample size, participant characteristics (including age, gender, Sokal score, and type of TKI used for treatment), duration of TKI treatment before discontinuation, duration of DMR before discontinuation, depth of molecular response (MR) before discontinuation of TKI, 12 months and 24 months the number of patients in treatment-free remission. Data collection was performed using Excel, with one researcher extracting data from the included studies and another researcher confirming the accuracy of the data. Any disagreements were resolved by consensus.

2.5 Risk of *bias* (quality) assessment

Two reviewers (Zheng AND Tang) independently assessed the risk of bias of the included studies. Included non-randomized clinical studies were assessed using the MINORS scale [20]. Any disagreement will be resolved by mutual discussion as well as expert review. Publication bias was assessed by Egger's linear regression test.

2.6 Statistical analysis

We obtained the required data from the original article or from the relevant Kaplan–Meier curves provided in the original article and calculated the ratio of patients in TFR for each study and the 95% confidence intervals (CI) from the extracted data in conjunction with Engauge Digitizer 4.1 and an excel file [21] provided by Tierney et al., and stabilized the variance of the studies by using the Freeman-Tukey double cosine transformation.

The appropriateness of summarizing the results of individual studies was assessed using the I^2 statistic, which determines statistical heterogeneity. The I^2 value reflects the total variability of the various studies as indexed by the study population, treatment regimen, or discontinuation, rather than chance or random error. $I^2 < 30\%$ was defined as mild heterogeneity, $30\% < I^2 < 50\%$ was defined as moderate heterogeneity and $> 50\%$ was defined as high heterogeneity. A fixed-effects model will be used for calculations when $I^2 < 50\%$; a random-effects model will be used for calculations of combined effect values (overall mean TFR rate) and 95% confidence intervals (CI) when $I^2 \geq 50\%$.

Subgroup analyses will be used to examine sources of heterogeneity and to determine whether the overall effect of the included trials varies with the type of trial and clinical characteristics such as year of publication, depth of molecular response before discontinuation, and molecular relapse criteria. type of TKI, prior interferon therapy, age, gender, Sokal score, the median duration of DMR, and median duration of TKI therapy.

The above processing was performed using the "metaprop" procedure of "meta" package of Stata17.0 software. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Study identification

Our initial search yielded 6938 studies, 4369 were excluded due to duplication, and after initial screening of titles and abstracts, 2026 were further excluded due to incompatibility with the topic and other language. The remaining 533 articles were further screened, and 514 were excluded because of (1) Reviews or meta-analyses; (2) Case reports, Replies,

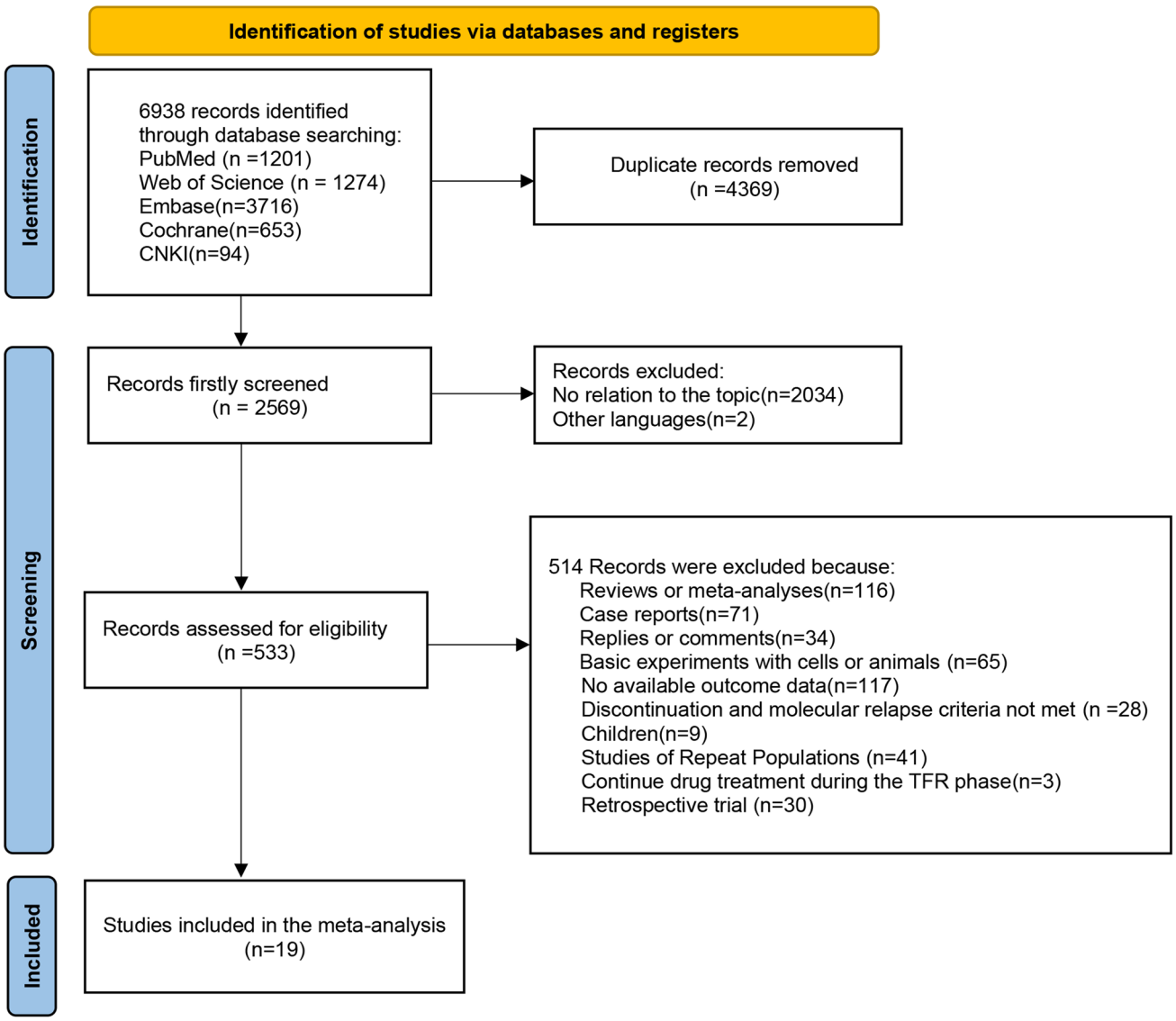

Fig. 1 Flow chart of the single-arm meta-analysis

or comments; (3) Lack of required TFR rate; (4) Pediatric patients; (5) Retrospective trials; (6) Basic experiments with cells or animals. The concrete selection procedure is depicted in Fig. 1.

Only one discontinuation-related RCT has been published since the establishment of the library, but it was excluded due to non-compliance with the discontinuation criteria [22]. The trial by Sung-Eun Lee and his colleagues in 2013 had a 100% TFR in transplanted patients and did not analyze the transplantation group separately [23], and the D-FREE trial was aborted after a one-year TFR of 16.7%, probably due to the insufficient duration of TKI treatment and insufficient duration of the DMR [24]. They were excluded to minimize the impact on data synthesis. The HOVON and the NILST trial defined molecular relapse criteria as a single loss of MR 4.5 [22, 25], and the STIM molecular relapse criterion of a single loss of MR 5.0 were excluded [12].

3.2 Study characteristics

A total of 19 trials [26–44] involving 2336 patients were included in this meta-analysis. Eight trials have investigated the probability of TFR after imatinib treatment [26, 28, 35, 39, 42–44], four trials have investigated the probability of TFR after dasatinib treatment [27, 31–33], three trials have investigated the probability of TFR after nilotinib treatment [29, 37, 40], and the remaining four trials have investigated the probability of TFR after treatment with different TKIs [30, 34, 36, 41]. There were no clinical trials related to the discontinuation of flumatinib, third-generation TKIs, etc., and only the LAST, GIMEMA CML0307 trial had 7 patients on bosutinib [29, 30].

The discontinuation criteria for all trials was maintaining a stable DMR. All included trials defined molecular relapse as loss of MMR/loss of MR4.0 and as a trigger for restarting the TKI. Additional detailed characteristics of the included trials are shown in Tables 1 and 2.

Major molecular response (MMR) was defined as BCR-ABL/ABLIS ≤ 0.1% by QPCR. MR4 was defined as BCR-ABL/ABLIS ≤ 0.01% (internal reference ABL copy number ≥ 10^4); MR4.5 was defined as BCR-ABL/ABLIS ≤ 0.003 2% (internal reference ABL copy number ≥ 3.2×10^4); MR5 was defined as BCR-ABL/ABLIS ≤ 0.001% (internal reference ABL copy number ≥ 10^5) and/or BCR-ABL was not detected (i.e., UMRD/CMR). DMR was defined as obtaining the efficacy of MR4 and above. TFR was defined as the period when TKI therapy was stopped and no molecular relapse occurred [7].

3.3 Risk of *bias* (quality) assessment

To assess the quality of non-comparative single-arm clinical trials, we used the MINORS Methodological items. Specifics regarding the quality evaluation are included in Table 3. The inclusion of trials had no publication bias as obtained by Egger's linear regression test (P = 0.124), which is shown in Fig. 2.

3.4 Treatment-free remission rate and safety

Using random-effects modeling combined, the overall mean TFR rate at 12 months was 59% (95CI: 0.56–0.63; $I^2 = 61\%$) and the overall mean TFR rate at 24 months was 55% (95CI: 0.52–0.59; $I^2 = 58\%$) with a high heterogeneity, as shown in Figs. 3 and 4.

Of the 19 trials included in this review, no CML-related progressions or deaths were reported during the TFR period, and only one case of disease progression was reported in the A-STIM trial [43]. This patient was retreated with imatinib after loss of MMR but suddenly progressed into the blast phase in the third month after regaining CMR**.** Therefore, it was not yet possible to consider that a direct relationship exists between this case and TKI discontinuation.

The cumulative incidence of adverse reactions during the TFR are detailed in Table 2. Adverse reactions were mainly withdrawal syndromes with clinical manifestations of skeletal connective tissue pain. We do not synthesize the data here because most recent trials have not documented adverse reactions.

3.5 Subgroup analyses

3.5.1 Previous interferon therapy

As shown in Fig. 5, the median proportion of patients previously treated with interferon across trials was 12%. The overall mean 24-month TFR rates for trials with a proportion of previously IFN-treated patients ≤ 12% [27, 31, 32, 36, 39, 40] and

Table 1 Basic characteristics of included studies

Study	Author	Year	Register number	N	Multicenter	Depth of MR required	MolRec	Monitor method	No. of patients in TFR at 12 m (%)	No. of patients in TFR at 24 m (%)
TWISTER	David M. Ross	2013	ACTRN12606000118505	40	YES	MR5	MMR***	RQ-PCR	21 (52.5)	18 (45.0)
A-STIM	Philippe Rousselot	2014	NR	80	YES	MR5	MMR	RQ-PCR	51 (63.7)	51 (63.7)
ISAV	Silvia Mor	2015	NCT01578213	108	YES	MR5	MMR	RQ-PCR	67 (62.0)	58 (51.9)
ENESTfreedom	A Hochhaus	2016	NCT01784068	190	YES	MR4.5	MMR	RQ-PCR	98 (51.6)	93 (48.9)
STOP 2G-TKI	Delphine Rea	2016	IRB 00006477	60	YES	MR4.5	MMR	RQ-PCR	38 (63.3)	35 (58.3)
KID	Sung-Eun Lee	2016	NCT01564836	90	YES	MR5	MMR	RQ-PCR	56 (62.2)	53 (58.8)
JALSG-STIM213	Naoto Takahashi	2017	UMIN000011971	68	YES	MR4.5	MMR	IS-PCR	46 (67.6)	46 (67.6)
ENESTop study	Francois-Xavier Mahon	2018	NCT01698905	126	YES	MR4.5	MR4	RQ-PCR	73 (57.9)	67 (53.2)
EURO-SKI	Susanne Saussele	2018	NCT01596114	755	YES	MR4	MMR	RQ-PCR	404 (53.5)	378 (50.1)
DOMEST	Shin Fujisawa	2018	UMIN000012472	99	YES	MR4	MR4	RQ-PCR	68 (68.6)	64 (64.6)
STAT2	Naoto Takahashi	2018	UMIN000005904	78	YES	MR4.5	MMR**	RQ-PCR	53 (67.9)	49 (62.8)
DADI	Shinya Kimura	2020	UMIN000011099	58	YES	MR4-4.5	MMR*	RQ-PCR	32 (55.1)	32 (55.1)
D-STOP	Takashi Kumaga	2020	NCT01627132	54	YES	MR4	MR4	RQ-PCR	34 (63.0)	32 (59.3)
D-NEWS	Hiroki Yamaguchi	2020	NCT01887561	26	YES	MR4	MR4	RQ-PCR	10 (38.5)	10 (38.5)
LAST	Ehab Atallah	2021	NCT02269267	171	YES	MR4	MMR	RQ-PCR	114 (66.7)	104 (60.8)
STIM2	Stéphanie Dulucq	2022	NCT#0134373	199	YES	MR4	MMR	ddPCR	107 (53.8)	93 (46.7)
GIMEMA CML 0307	Gabriele Gugliotta	2022	NCT00481052	24	YES	MR4	MMR	RQ-PCR	20 (83.3)	18 (75.0)
DASFREE	Neil P. Shah	2023	NCT01850004	84	YES	MR4.5	MMR	RQ-PCR	41 (48.7)	39 (46.4)
IMA-FREE	Deepak Goni	2023	CTRI/2018/08/015357	26	NO	MR4	MMR	RQ-PCR	12 (46.1)	11 (42.3)

NR: no report. MolRec: Molecular Relapse Criteria

*: loss of MMR or Loss of MR4 twice consecutively

**: loss of MMR or Loss of MR4.5 twice consecutively

***: loss of MMR or two consecutive positive screens

Table 2 Basic characteristics of included patients

Study	TKIs treatment	Treatment history	Age	Sex(male/female)	Sokal (%)				Duration of DMR before TKI discontinuation(months)	Duration of TKI therapy(months)	Interferon treatment	Adverse reaction
					Low	Intermediate	High	Unknown				
DASFREE	DAS	1st/2nd/3rd line	52	47/37	54	24	5	1	28.9	69.2	0%	11%
IMA-FREE	IMA	NR	57	12/14	16(61.5)	8(30.8)	2(7.7)	0	51	72	NR	NR
STIM2	IMA	1st line	NR	NR	82	74	30	13	NR	NR	NR	NR
GIMEMA CML 0307	NIL	1st line	62	11/13	14(58.3)	7(29.2)	3(12.5)	0	74	88	NR	NR
LAST	1G/2G TKIs	1st/2nd/3rd line	60	89/82	33	19	7	113	56.6	82.7	NR	NR
DADI	DAS	1st line	62.5	20/38	43(74.1)	14(24.1)	1(1.7)	0	23.3	NR	0%	NR
D-STOP	DAS	NR	56	32/22	24	11	6	13	51	92	0%	5.60%
D-NEWS	DAS	1st line	60.5	9/17	14(53.8)	11(42.3)	1(3.8)	0	NR	32.8	NR	21.2%
ENESTop study	NIL	2nd line	56	56/70	NR	NR	NR	NR	19.8	87.7	NR	74%
EURO-SKI	1G/2G TKIs	1st/2nd line	60	396/352	259	197	128	171	56.4	90	12%	31%
DOMEST	IMA	NR	62	65/34	56(56.1)	34(34.3)	9(9.1)	0	55	100	16%	NR
STAT2	IMA/NIL	2nd line	57	45/33	44(56.4)	17(21.8)	16(20.5)	0	NR	99	15%	14.1%
JALSG-STIM213	IMA	1st line	55	42/26	51(75)	6(8.8)	11(16.2)	0	NR	97.5	19%	15%
ENESTfreedom	NIL	1st line	55	NR	62	50	28	50	30.4	43.5	0%	8.4%
STOP 2G-TKI	2G TKIs	1st/2nd/3rd line	60	22/38	32	16	9	3	29	76	28%	NR
KID	IMA	1st line	56.2	38/52	29	23	15	23	39.9	80.8	9%	30%
ISAV	IMA	1st line	49	64/44	40	29	8	31	25.8	103.1	33%	21.20%
A-STIM	IMA	NR	55	40/40	41	22	16	1	41	79	52%	NR
TWISTER	IMA	1st line	58(62)*	19/21	20(50)	16(40)	4(10)	0	30(41)*	70(72)*	53%	NR

NR no report. *: Non-IFN treatment group and previous IFN treatment group, respectively

Table 3 Quality assessment of the non-comparative single-arm clinical trials included in the meta-analysis

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Score*
Silvia Mor	2	2	2	2	0	2	2	1	13
A Hochhau	2	2	2	2	0	2	2	0	12
Delphine Rea	2	2	2	2	0	2	2	2	14
Sung-Eun Lee	2	2	2	2	0	2	2	2	14
Takashi Kumagai	2	2	2	2	0	2	2	2	14
David M. Ross	2	2	2	2	0	2	2	2	14
Shin Fujisawa	2	2	2	2	0	2	2	2	14
Shinya Kimura	2	2	2	2	0	2	2	1	13
Naoto Takahashi	2	2	2	2	0	2	2	0	12
Naoto Takahash	2	2	2	2	0	2	2	2	14
Philippe Rousselot	2	2	2	2	0	2	2	2	14
Franc¸ ois-Xavier Mahon	2	2	2	2	0	2	1	0	11
Neil P. Shahl	2	2	2	2	0	2	2	2	14
Susanne Saussele	2	2	2	2	0	2	2	2	14
Stéphanie Dulucq	2	2	2	2	0	2	2	2	14
Deepak Goni	2	2	2	2	0	2	2	1	13
Gabriele Gugliotta	2	2	2	2	0	2	2	0	12
Ehab Atallah	2	2	2	2	0	2	2	1	13
Hiroki Yamaguchi	2	2	2	2	0	2	2	2	14

Numbers Q1-Q8 in heading signified:

Items Q1-Q8: Q1: A clearly stated aim; Q2: Inclusion of consecutive patients; Q3: Prospective collection of data; Q4: Endpoints appropriate to the aim of the study; Q5: Unbiased assessment of the study endpoint; Q6: Follow-up period appropriate to the aim of the study; Q7: Loss to follow up less than 5%; Q8: Prospective calculation of the study size

*The items are scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate)

Q1: A clearly stated aim: the question addressed should be precise and relevant in the light of available literature. Q2: Inclusion of consecutive patients: all patients potentially fit for inclusion (satisfying the criteria for inclusion) have been included in the study during the study period (no exclusion or details about the reasons for exclusion). Q3: Prospective collection of data: data were collected according to a protocol established before the beginning of the study. Q4: Endpoints appropriate to the aim of the study: unambiguous explanation of the criteria used to evaluate the main outcome which should be in accordance with the question addressed by the study. Also, the endpoints should be assessed on an intention-to-treat basis. Q5: Unbiased assessment of the study endpoint: blind evaluation of objective endpoints and double-blind evaluation of subjective endpoints. Otherwise the reasons for not blinding should be stated. Q6: Follow-up period appropriate to the aim of the study: the follow-up should be sufficiently long to allow the assessment of the main endpoint and possible adverse events. Q7: Loss to follow up less than 5%: all patients should be included in the follow up. Otherwise, the proportion lost to follow up should not exceed the proportion experiencing the major endpoint. Q8: Prospective calculation of the study size: information of the size of detectable difference of interest with a calculation of 95% confidence interval, according to the expected incidence of the outcome event, and information about the level for statistical significance and estimates of power when comparing the outcomes

*The items are scored 0 (not reported), 1 (reported but inadequate) or 2 (reported and adequate)

for trials with a proportion > 12% [34, 35, 38, 41–44] were 51% (95CI: 0.48–0.54) and 60% (95CI. 0.55–0.65) respectively, with statistical difference (P = 0.003). These data suggested that better TFR was associated with prior interferon therapy.

3.5.2 Depth of Molecular Response Before Entering the TFR Phase

As shown in Fig. 6, the overall mean 24-month TFR rates for reaching molecular reaction depth MR4.5 [27, 34, 37, 38, 40, 41] and reaching MR5.0 [34, 38, 39, 42–44] in each trial were 51% (95CI: 0.46–0.55) and 62% (95CI: 0.53- 0.69) respectively, with statistical difference (P = 0.020). Where STAT2 and STIM213 counted the number of patients who achieved MR5 and MR4.5, respectively, in subgroups we analyzed them separately. These data revealed that better TFR was associated with the depth of molecular response.

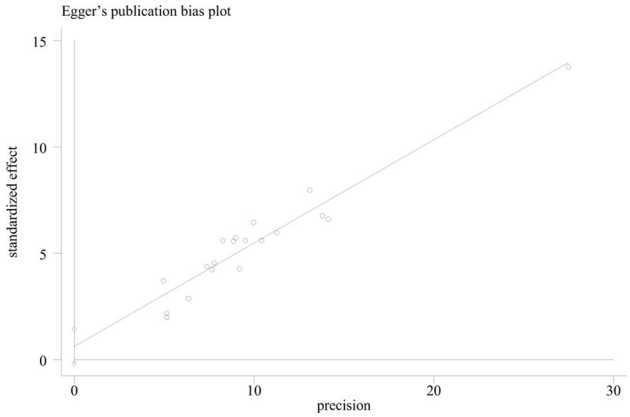

Fig. 2 Egger's linear regression test of publication bias

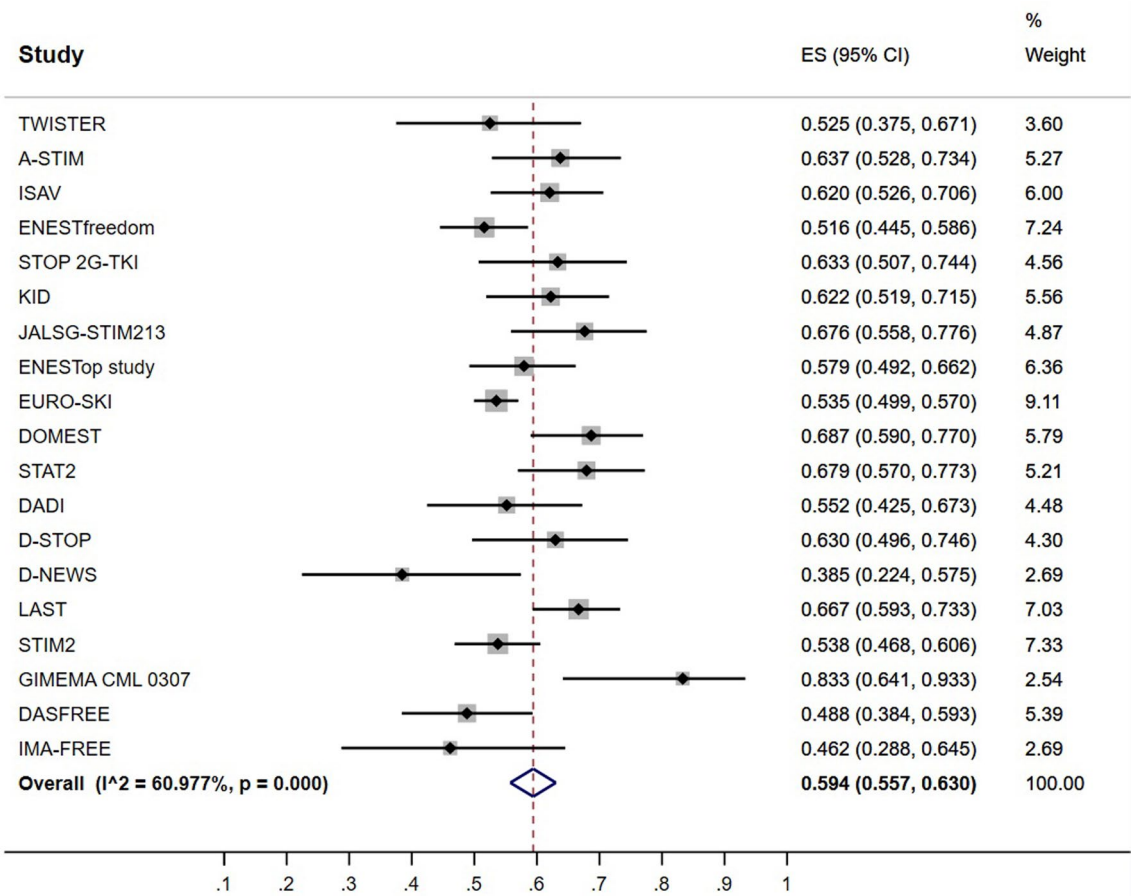

Fig. 3 Forest plot of the overall mean TFR rate at 12 months after TKI discontinuation. TFR, treatment-free remission, TKI, tyrosine kinase inhibitor, 95% CI, 95% confidence interval

3.5.3 Other factors

No significant differences were found in the 24-month TFR when subgroup analyses were conducted on other factors, such as the year of trial publication, molecular relapse criteria, and patient characteristics (TFR (supplementary Fig. 1), median duration of DMR (supplementary Fig. 2), gender (supplementary Fig. 3), age (supplementary Fig. 4), Sokal score (supplementary Fig. 5), depth of molecular reaction (MR) (supplementary Fig. 6), TKI type (supplementary Fig. 7), publish year before TKI discontinuation (supplementary Fig. 8) and molecular relapse criteria (supplementary

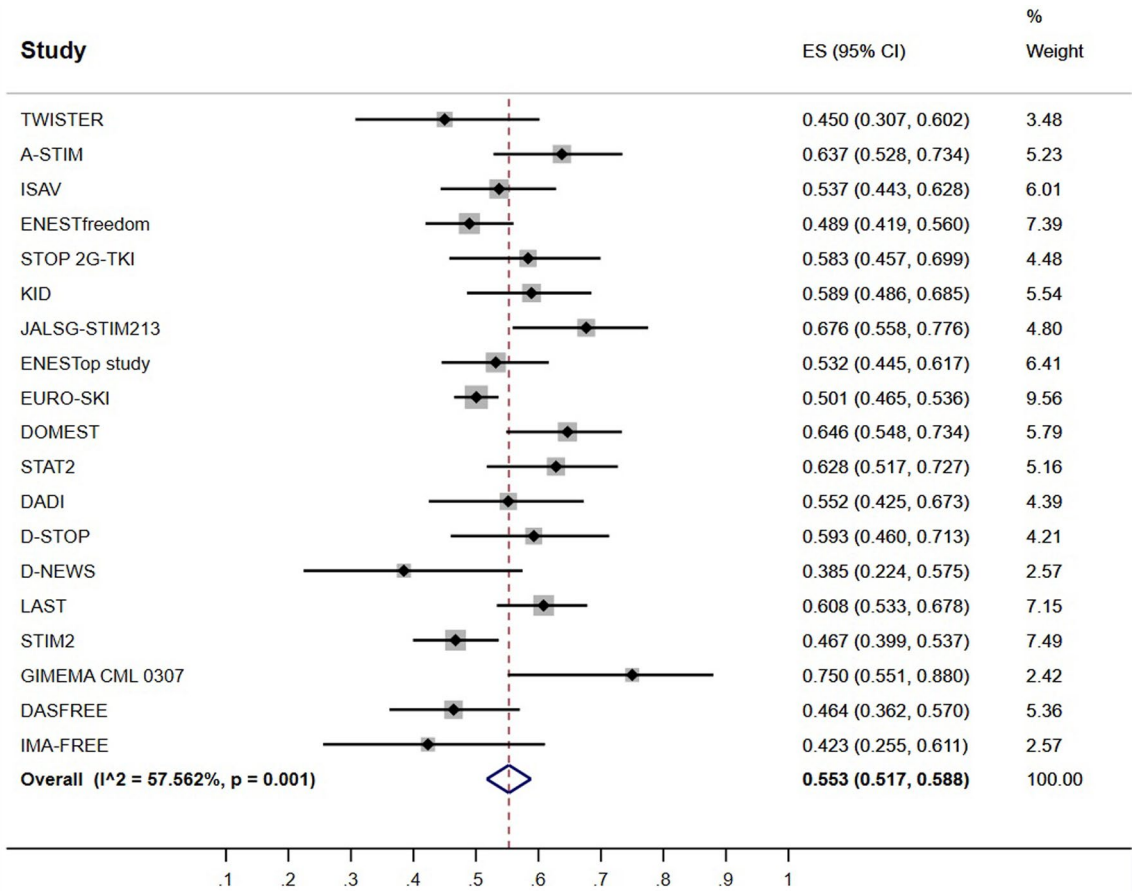

Fig. 4 Forest plot of the overall mean TFR rate at 24 months after TKI discontinuation. TFR, treatment-free remission, TKI, tyrosine kinase inhibitor, 95% CI, 95% confidence interval

Fig. 9). Our findings indicated that these other factors were not associated with TFR rates. The above results were shown in Supplementary figures and the results of subgroup analyses by stata17.0.

4 Discussion

Our study shared a similar statistical methodology and subgroup analysis strategy to the previous study [17]. However, we had two highlights that distinguish it from the previous study. Firstly, our inclusion criteria were strictly limited to prospective clinical trials. Secondly, we integrated population characteristics and trial characteristics in the subgroup analyses to provide a more comprehensive and insightful analysis. Here, we showed a 12-month overall mean TFR rate of 59% [95CI:0.56–0.63] and a 24-month overall mean TFR rate of 55% [95CI:0.52–0.59], indicating that more than half of the CML-CP patients were able to maintain TFR two years after TKI discontinuation. Our subgroup analysis showed that better TFR was associated with prior interferon therapy (24 months: P = 0.003), and molecular response depth MR5.0 (24 months: P = 0.02), which was in line with the findings of previous trials [26, 36, 38], although this analysis existed heterogeneity.

Interestingly, though several trials and our study have identified interferon as a potential positive prognostic factor for TFR, other studies pointed out that a prior IFN treatment and/or duration of IFN therapy may be associated with a lower risk of MR [43–45]. The recent TIGER trial randomized newly diagnosed patients to receive either nilotinib alone or in combination with Peg-Interferon (Peg-IFN) and Peg-IFN Maintenance Therapy. However, the combination of nilotinib and IFN did not significantly increase the probability of achieving long-term TFR during the discontinuation phase [46]. There was a high degree of heterogeneity in the included trials, with most of them recording only the number of people using interferon, making a detailed description of interferon exposure difficult. Given that patients will mainly be treated with TKI monotherapy in the future, the effect of interferon on TFR remains to be explored.

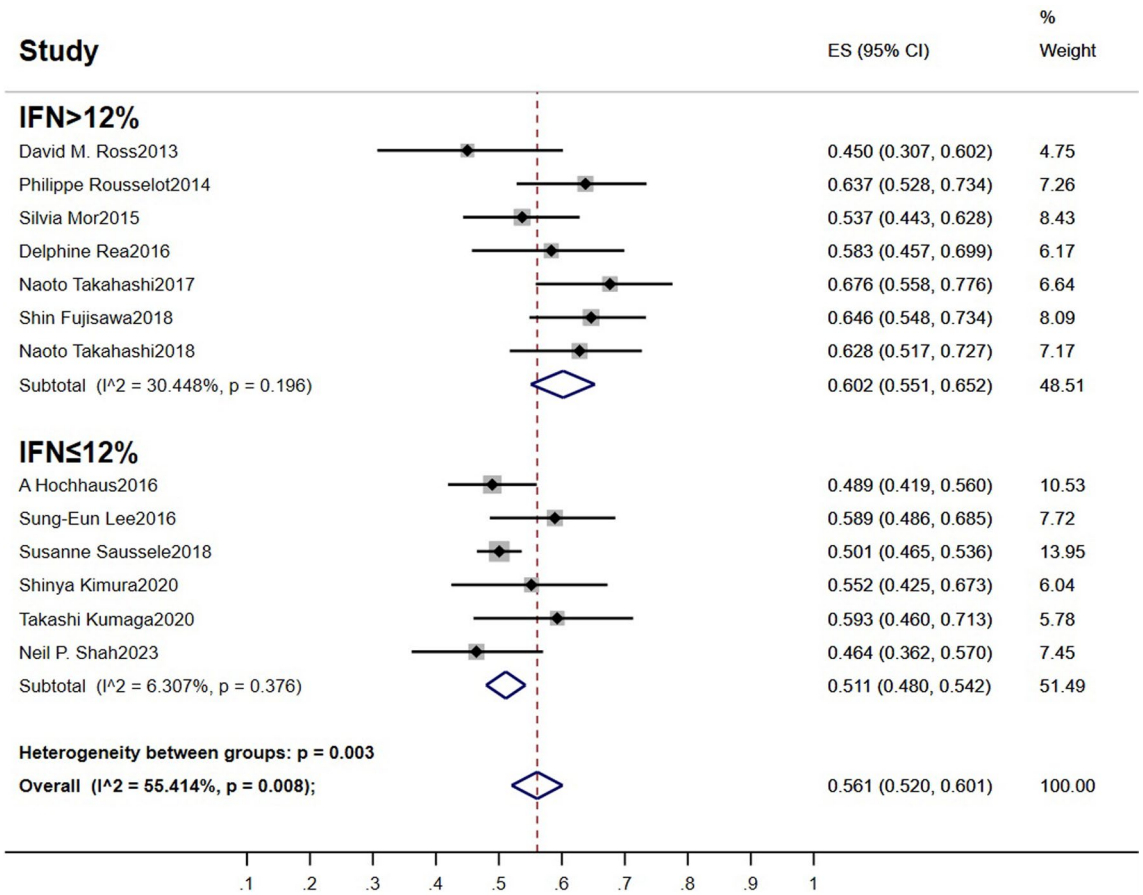

Fig. 5 Subgroup analysis for TFR rate according to previous interferon therapy before TKI discontinuation. TFR, treatment-free remission, TKI, tyrosine kinase inhibitor, 95% CI, 95% confidence interval

According to the previous study [17], the duration of DMR and TKI therapy before discontinuation were found to be associated with TFR. However, our analysis was not, which is consistent with the results of subgroup analyses of several trials, where the TFR rate was not associated with the duration of TKI treatment and the duration of DMR before TKI discontinuation [31, 32, 34, 41, 42]. One possible explanation was the effect of heterogeneity in the inclusion of trials with other factors, further research may significantly impact the estimated effect sizes and potentially alter them. Another possible explanation is that the TFR rate depends mainly on the patient's sensitivity to the TKI rather than the duration of treatment, i.e., the depth of the molecular response obtained after treatment with a TKI, and the same factor is also responsible for the time required to reach that depth after TKIs treatment, which has not been recorded in the majority of the trials, and which would be a predictor worth investigating. This factor was reported in only two trials, the TWISTER trial showed an association with better TFR rate (P = 0.04), but the KID trial did not correlate with TFR rate (P = 0.152) [39, 44]. In the study by Naranie Shanmuganathan and his colleagues, time to BCR-ABL1 halving on TKI treatment was the strongest independent predictor of TFR maintenance [47].

Adverse effects in patients maintained on TFR were mainly characterized by skeletal muscle myalgia, i.e., withdrawal syndrome. Although the EURO-SKI and ENESTop study reported rates of 31% and 74%, the rates of grade 3 to 4 adverse reactions were only 1% and 13%, respectively [36, 37]. On the other hand, the D-STOP trial reported an incidence of only 5.6% [31]. In addition, no CML-related progressions or deaths were reported during the TFR period. The above demonstrates the feasibility and safety of TKI discontinuation.

The following limitations are worth considering in this meta-analysis.

Firstly, there was a high degree of heterogeneity in the clinical trials we included. This arose from differences in trial design, including criteria for the discontinuation population, the definition of molecular relapse, and different molecular monitoring methods such as Droplet digital PCR (dd-PCR) and RQ-PCR. Dd-PCR demonstrated more accurate molecular relapse monitoring than RQ-PCR [48, 49]. Since there was no RCT for clinical trials related to drug discontinuation, it also

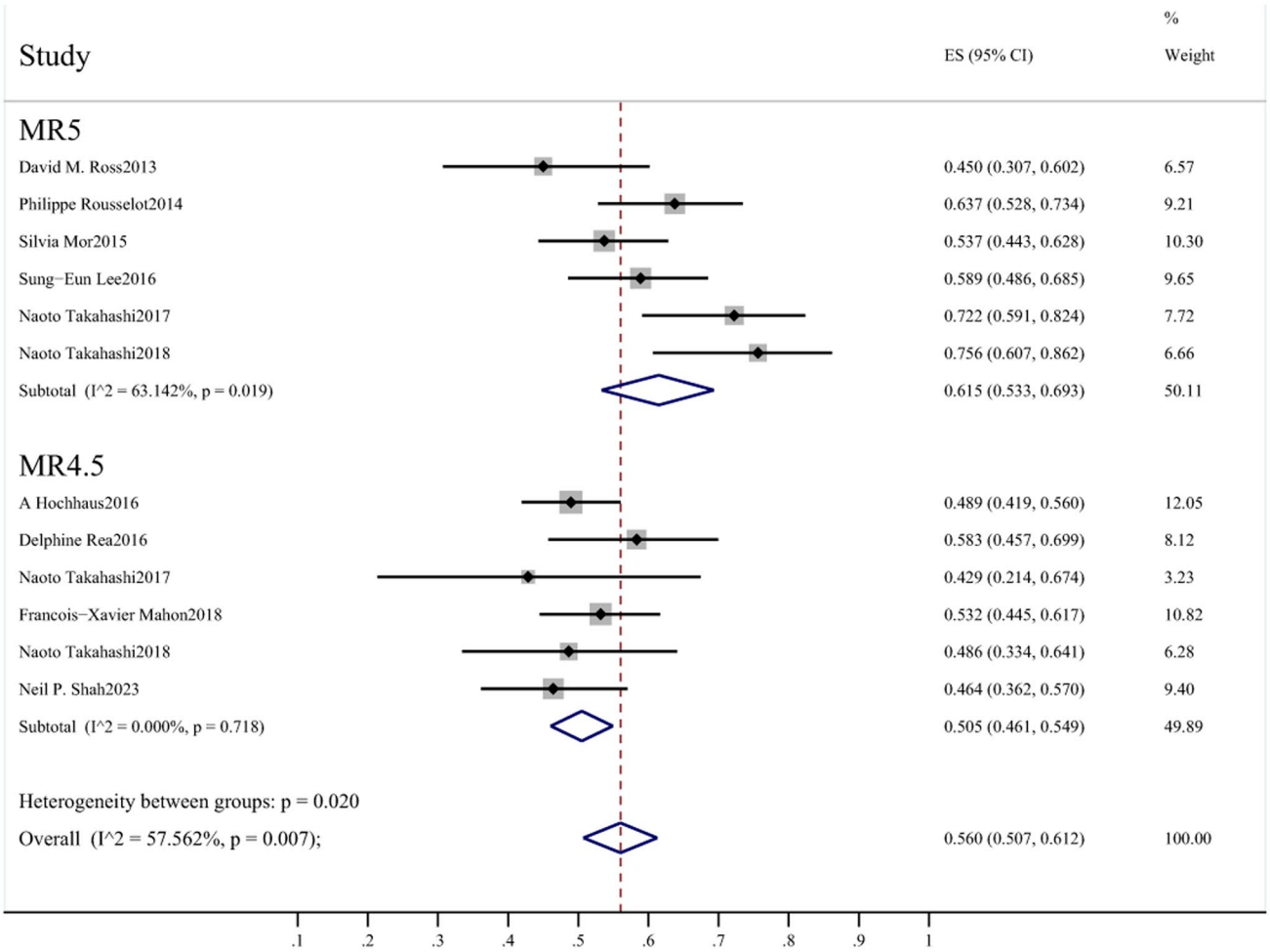

Fig. 6 Subgroup analysis for TFR rate according to the depth of molecular response before TKI discontinuation. TFR, treatment-free remission, TKI, tyrosine kinase inhibitor, 95% CI, 95% confidence interval

affected the magnitude of heterogeneity. Despite differences in trial design, the trials we included were of high methodological quality. To explore heterogeneity, our analyses were validated by different subcomponents.

Subsequently, we excluded retrospective clinical trials to avoid high heterogeneity and publication bias. A total of 30 retrospective studies were excluded in the rescreening, and we read these articles, which involved a large number of patients and had mostly high second-year TFR, such as the retrospective cohort study by M. Ansuinelli et al., with a second-year TFR of 74% [50]. Therefore, it would be of interest to analyze the discontinuation population of retrospective studies separately in the future.

In addition, we had not yet analyzed distant relapse. In clinical trials with sufficiently long follow-up, the TFR rate in the fifth year was 44% for DASFREE, 49% for EURO-SKI in the third year, and 53.57% for ENESTfreedom in the fourth year, et al. [27, 51, 52]. We noted the presence of distant recurrence, but there was a temporary lack of sufficient trial sample sizes for analysis. Similarly, the limited number of prospective trials included in this meta-analysis resulted in a low number of trials in certain subgroups in the subgroup analysis.

Self-renewing leukemic stem cells are associated with relapse, and some patients are still unable to achieve TFR due to the persistence of leukemic stem cells (LSCs) [53]. Paola Pacelli and his colleagues noted that despite fluctuating residual CD26 + LSCs, maintaining a stable TFR is still possible [54]. Namely, factors other than the presence of LSCs play a positive role in disease recurrence. A trial by M Matsushita et al. found a relapse rate of 63.6% in cxorf48-specific ctl-negative patients and 0% in cxorf48-specific ctl-positive patients [55]. Yazad D Iraniand and his colleagues' results showed that TIM-3 was consistently up-regulated in CD4 + T-cells, CD8 + T-cells, and T-regs in relapsed patients, compared to patients with maintained TFR. regs cells were consistently upregulated [56]. In addition, Gene expression analyses of publicly available datasets revealed increased expression of TIM-3 on CML stem cells in comparison to normal hematopoietic

stem cells. These results from recent studies of TKI discontinuation suggest that CXorf48 and TIM-3 may be promising therapeutic targets for durable treatment-free remission in immunotherapy for CML patients (Table 4).

Management of patients who discontinued TKIs should also focus on their psychological as well as other domains. Giora Sharf investigated that 56% of patients reported fear or anxiety during treatment discontinuation, and 60% reported withdrawal symptoms at the time of discontinuation [57]. Possible reasons for anxiety in patients who discontinue may be due to the uncertainty of relapse, adverse effects, and regular monitoring after discontinuation. The study [17] has shown that patients who failed TFR mostly resumed DMR after re-treatment with TKI. For molecular monitoring after TKIs discontinuation, the expert consensus of the European Leukemia Net proposes lifelong [7]. Our observation that TFR failure occurs mainly within one year, but a few patients still experience distant relapse, albeit slow onset [27, 51, 52], suggests that there is an urgent need for a cost-effective and user-friendly method for monitoring molecular response after discontinuation, as well as for more accurate predictors. Dennis Dong Hwan Kimand and his colleagues'

Table 4 Subgroup analysis of predictors of successful TFR

Variable	No. of trials	TFR rate at 24 months(95%CI)	P-value
Age			0.806
≤ 57	10	0.56(0.51–0.61)	
> 57	8	0.55(0.49–0.61)	
Sex			0.500
Male ≤ female	10	0.55(0.51–0.60)	
Male > female	8	0.58(0.52–0.64)	
Sokal score high			0.229
< 10%	4	0.53(0.40–0.66)	
≥ 10%	4	0.63(0.52–0.73)	
Sokal score low			0.523
> 56.6%	4	0.61(0.48–0.72)	
≤ 56.6%	4	0.55(0.43–0.66)	
Type of TKI therapy			
Ima	8	0.56(0.50–0.63)	Ima vs Das:0.309
Das	4	0.51(0.43–0.59)	Ima vs Nil:0.902
Nil	3	0.55(0.45–0.66)	Das vs Nil:0.499
Interferon therapy			0.003
≤ 12%	6	0.51(0.48–0.54)	
> 12%	7	0.60(0.55–0.65)	
Duration of DMR before TKI discontinuation			0.096
≤ 40 m	8	0.52(0.49–0.56)	
> 40 m	7	0.59(0.52–0.66)	
Duration of TKI therapy before TKI discontinuation			0.164
≤ 82.7 m	9	0.53(0.48–0.59)	
> 82.7 m	8	0.59(0.53–0.65)	
Depth of MR before TKI discontinuation			0.020
MR4.0	8	0.55(0.48–0.61)	MR4 vs MR5:0.203
MR4.5	6	0.51(0.46–0.55)	MR4 vs MR4.5:0.282
MR5.0	6	0.62(0.53–0.69)	MR5 vs MR4.5:0.020
Publish year			0.631
< 2019	11	0.57(0.52–0.61)	
≥ 2019	8	0.55(0.49–0.61)	
MolRec			0.963
Loss MMR	12	0.56(0.52–0.60)	
Non-loss MMR	7	0.56(0.50–0.62)	

MolRec Molecular Relapse Criteria

study suggest that BCR-ABL1 transcriptional doubling time can be used as a predictor of TFR failure in CML-CP patients after imatinib discontinuation, potentially avoiding the need for frequent monthly monitoring of molecular assays [58].

The results of our analysis suggest a strategy that seems feasible to improve the success of TFR by attempting to rationalize the application of interferon before or after discontinuation of the drug for those who reach a molecular response depth of MR 5. A trial by Burchert and his colleagues demonstrated that the strategy of IFN/imatinib induction therapy followed by temporary IFN maintenance therapy improves long-term TFR rates**.** After a median follow-up of 7.9 years, the TFR rate for patients who reached MR4/MR4.5 when imatinib was discontinued was 84% [59].

Another strategy that seems feasible is dose-decreasing discontinuation. A retrospective analysis in Italy found that low-dose TKIs do not seem to affect the likelihood of achieving DMR, but may even improve TFR rates, which were 74% in the second year [60]. The DESTINY trial demonstrated that discontinuation of TKIs after one year of dose halving resulted in a 72% recurrence-free survival rate in the second year [61]. Still, more trials are needed to validate whether attempted discontinuation after dose reduction promotes TFR.

The number of RCTs and prospective clinical trials related to this area is limited, and more high-quality clinical trials are needed to further explore this. Meanwhile, trials of other second and third-generation TKIs are anticipated. The latest asciminib trial demonstrated superior efficacy and fewer adverse events [62]. We searched ClinicalTrials.gov and found that there are still multiple large trials with no results output, and we will update our opinion if we find an impact on predictors in the future.

5 Conclusion

Our study showed that CML-CP patients who reached DMR could achieve a TFR of 59% in the first year and 55% in the second year after TKIs discontinuation, while achieving a molecular response depth of MR5.0 or higher and prior interferon therapy were associated with higher TFR rates. Further validation of the above predictors is needed due to the heterogeneity of the included trials, while we hope that future trials will focus on the time taken for patients to enter the DMR and the emergence of withdrawal syndromes.

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Author contributions Zhenxiang Zheng and Zhao Yin conceived and designed the experiments. Jie Zhou and Hao Tang analyzed and interpreted the data. Xinxia Zhang performed the experiments. Yangmin Zhu and Liling Zheng drafted the article and all other authors revised the article. All authors approved the final version of the article.

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Data availability Data can be obtained upon request.

Declarations

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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