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Safety and efficacy of Combination of Fludarabine, Cyclophosphamide, and Rituximab compared to Chemotherapy alone in Chronic Lymphocytic Leukemia Patients

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Abstract
Background: Chronic lymphocytic leukemia, one of the most common hematological malignancies, has been treated with several treatment regimens. Recently chemoimmunotherapy with Fludarabine, Cyclophosphamide, and Rituximab (FCR) regimen have shown significant success in treating these patients.
Aim: This systematic review aims compare the efficacy and safety of chemoimmunotherapy combination (FCR) to chemotherapy alone (FC) in chronic lymphocytic leukemia patients.
Methods: Three healthcare related databases were systematically searched: PubMed, Cochrane registry for clinical trials, and cumulative index for nursing and allied health literature (CINAHL) with appropriate combination of keywords and medical subject headings (MeSH). Then, duplicate citations were removed from the search result followed by Title/Abstract screening. After that, these are articles underwent full text screening based on the inclusion and exclusion criteria. Data were extracted regarding overall survival (OS), progression free survival (PFS), objective response rate (ORR), and serious adverse effects. For risk of bias analysis JADAD score was utilized.
Results: Total number six clinical trials were involved with total 3433 patients, 1723 patients in the experimental arm and 1710 patients in the control arm. Five out of six clinical trials reported higher progression free survival (PFS) in the experimental arm, four out of five clinical trials reported higher overall survival (OS) in the experimental arm, and four out of five clinical trials reported higher objective response rate (ORR) in the experimental arm. Majority of the clinical trials of good quality with JADAD score 3 or more for five clinical trials.
Conclusion: Chemoimmunotherapy with FCR is more effective than chemotherapy alone with FC regimen, however, toxicity is higher in the experimental arm. Future trials should focus on adverse effects.

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Keywords: Chronic Lymphocytic Leukemia, Rituximab, Chemoimmunotherapy, Fludarabine, FCR Regimen

Introduction

Chronic lymphocytic leukemia (CLL) is one of the most prevalent hematological malignancies in older people with 300 incidences per million population each year among those who are 80 years or older [1]. More importantly, the prevalence of CLL is increasing over time and the true prevalence is substantially higher than the data provided by any cancer registry as majority of the cases are less problematic at the beginning [2]. CLL presents with a variable clinical course with median overall survival is ranging from 18 months to 20 years and 5-year survival is more than 83%, however, survival is different for different stagesof CLL patients [3]. Although many patients in the early and asymptomatic stage may not require any treatment, however, symptomatic and recurrent CLL patients should receive treatment [4].

As suggested by European society of Medical Oncology (ESMO), there are several treatment options for CLL patients which should be guided by patient and disease characteristics known to influence therapeutic outcomes [5].

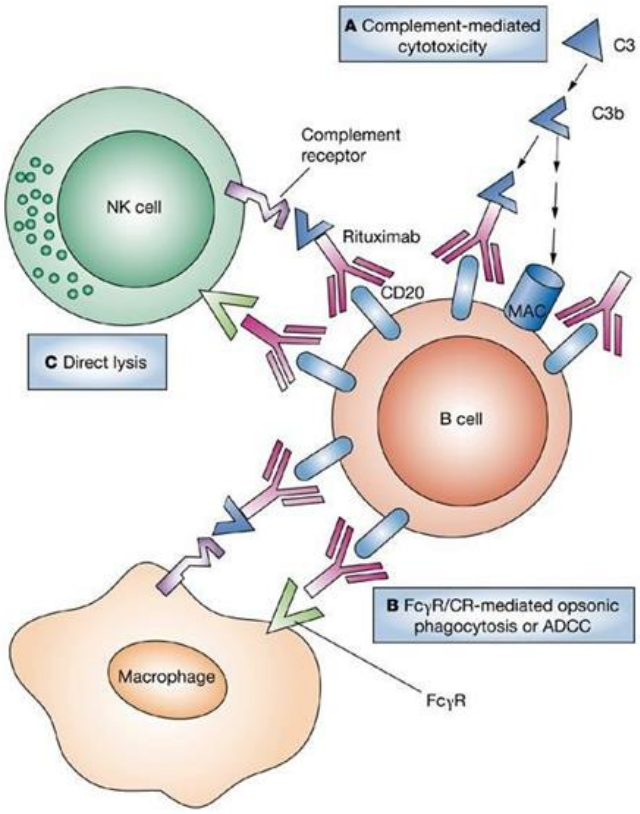

Fig 1: Rituximab binding to the CD20 ligand and binds NK cells, Macrophage, Antiboy causing the cell death by direct lysis, complement mediated cell cytotoxicity, and antibody dependent cell cytotoxicity [9].

Rituximab, a monoclonal antibody, is widely used in the management of CLL patients as this has shown efficacy in treating these patients [10]. Rituximab works by binding to the cluster of differentiation 20 (CD-20) by the variable portion of the monoclonal antibody, whereas the constant portion of the antibody binds to the macrophage, NK cells causing the death of the cell. This Rituximab can also cause the activation of the complement process, leading to the complement-mediated cell death, moreover, Rituximab can also attract other antibodies, causing more cell death by antibody-dependent cell- mediated cytotoxicity (ADCC) [9].

Previously, CLL patients were treated by different regimens like Fludarabine and Rituximab (FR) combination, which reported extended survival (both PFS and OS) along with good response when treated concurrently or sequentially [11]. However, when FR regimen was compared to FCR regimen in Cancer and Leukemia Group B (CALGB) 10404 trial, this was reported that FCR regimen is superior to FR regimen regarding the OS, PFS, and ORR [12]. However, CALGB 10404 trial also reported higher adverse effect in the FCR regimens, specially hematologic toxicities, for example, leucopenia, thrombocytopenia, neutropenia, anemia and increased incidence of infectious disease [12].

Similarly, Bendamustine and Rituximab combination was also administered in the CLL patients with increased survival benefit compared to chemotherapy regimens. However, BR was compared to FCR in CLL10 study which reported higher PFS benefit in FCR receiving patients though there was no benefit in the OS [13]. Furthermore, CLL10 trial reported higher adverse effects in the FCR arm compared to the patients receiving BR regimen [13]. More importantly, this CLL10 trial reported higher rates of second malignancy among FCR recipients for CLL [14]. Although data suggested that second malignancy rate is similar in both concurrent and sequential treatment, it was hypothesized that concurrent treatment with FCR regimen causes more immunodeficiency, causing higher rates of second malignancy [15]. CLL10 even suggested that second malignancy rate increased for FCR recipients further in older patients (65 years or more in age) [13].

Management of CLL has progressed in a fast pace in the past 20 years, with the integration of CD20-based monoclonal antibodies, B cell lymphoma 2 (BCL 2)- targeted treatment, and B cell receptor (BCR) targeted treatment with improved progression free survival and higher response rates [16, 17].

Previous trials also reported higher toxicity among FCR recipients, making this a difficult treatment for patients with poor performance status or unfit patients [18]. The most common adverse effects were neutropenia and leucopenia, leading increased rates of common infections (bacterial, varicella zoster, and T lymphopenia related opportunistic infections), causing occasional postponement in treatment, resulting in potential of lower response and survival benefit [19]. To be more specific, 65 years or older patients suffered more incidence of infections from this FCR regimens with more than 10 percent incidence of grade 3 opportunistic infections [19].

It is widely recognized that FCR provides higher response rate and survival benefit for IGHV mutant, 11q or 17p deleted patients. However, in patients without these mutations (IGHV, 11q or 17p deletion) Ibrutinib seems to better treatment regimen though Ibrutinib is less efficient in IGHV mutant patients compared to FCR regimen [20]. To cover that Ibrutinib was added to the FCR regimen which increased the response in both IGHV mutated and non-mutated patients, however, toxicity was even higher in this regimen [21].

Two systematic reviews were conducted in this topic, however, none of these reviews included the broad CLL population treated with FCR chemoimmunotherapy regimen [22, 23]. One systematic review only focused on the unfit or elderly CLL patients which rather narrowed its vision and updated data were not included [23]. Another one systematic review included the updated data, however, this only included maintenance treatment with FCR regimen without even considering frontline or second line treatment [22].

This systematic review is quite important and relevant as this deals with CLL patients which is one of the most common hematological malignancy in the world with a large number of patients. More importantly, no single treatment is settled for CLL patients due to the conundrum regarding the high response versus higher toxicity in combination chemoimmunotherapy regimens. If this systematic review is successful, then might help to change the treatment paradigm in CLL patients.

This systematic review aims to assess the efficacy and safety of Combination of Fludarabine, Cyclophosphamide, and Rituximab (FCR0 compared to chemotherapy alone in chronic lymphocytic leukemia patients. To assess the efficacy of the treatment regimen OS, OFS, and ORR was compared. To evaluate the toxicity grade 3-4 toxicity was compared. To find out the risk of bias JADAD scored was

calculated and compared.

Methodology

The newly published Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline was followed for the detailed and transparent administration of the systematic review and reporting of the review [24]. Three databases were systematically searched as per the PRISMA guideline: PubMed, Cumulative Index of Nursing and Allied Health Literature (CINAHL), and Cochrane central registry for clinical trials.

PubMed search strategy

Five keywords were utilized for the PubMed search: Chronic lymphocytic leukemia, Rituximab, Fludarabine, Cyclophosphamide. Different commonly used synonyms were also used, for example, for chronic lymphocytic leukemia following synonyms were also used in the search: "Chronic B-Cell Leukemias", "B Lymphocytic Leukemia, Chronic", "Chronic B-Lymphocytic Leukemias", "Diffuse Well Differentiated Lymphocytic Lymphoma", "Chronic Lymphatic Leukemia", "Chronic Lymphatic Leukemias", "Leukemia, Chronic Lymphocytic", "Leukemia, Chronic Lymphocytic, B-Cell", "Leukemia, Lymphoblastic, Chronic", "Leukemia, Lymphocytic, Chronic", CLL et.. These different synonyms were used to ensure that no search results were excluded. These synonyms were added with the Boolean operator OR, after that, these separate search strings were combined with another Boolean operator AND. These lead to final search which is further focused by the used of limiters like "Human" as species, "English" as language and "Randomized controlled trial" as type of study. Finally, total search results were 28 studies, and the search was conducted 17th of November, 2022 (Table 1).

Table 1: PubMed search strategy

Keywords or MeSH	Search string	Number of results
Rituxumab	#1: Rituximab [Title/Abstract] OR "CD20 Antibody, Rituximab" [Title/Abstract] OR "Rituximab CD20 Antibody" [Title/Abstract] OR Mabthera [Title/Abstract] OR Rituxan [Title/Abstract]	25,960
Chronic lymphocytic leukemia	#2: "Chronic B-Cell Leukemias" OR "B Lymphocytic Leukemia, Chronic" [Title/Abstract] OR "Chronic B-Lymphocytic Leukemias" [Title/Abstract] OR "Diffuse Well Differentiated Lymphocytic Lymphoma" [Title/Abstract] OR "Chronic Lymphatic Leukemia" [Title/Abstract] OR "Chronic Lymphatic Leukemias" [Title/Abstract] OR "Leukemia, Chronic Lymphocytic" [Title/Abstract] OR "Leukemia, Chronic Lymphocytic, B-Cell" [Title/Abstract] OR "Leukemia, Lymphoblastic, Chronic" [Title/Abstract] OR "Leukemia, Lymphocytic, Chronic" [Title/Abstract] OR CLL [Title/Abstract]	17,617
Fludarabine	#3: Fludarabine [Title/Abstract] OR "fludarabine 5'-monophosphate" [Title/Abstract] OR "9 beta-D-arabinofuranosyl-2-fluoroadenine monophosphate" [Title/Abstract] OR "fludarabine monophosphate" [Title/Abstract]	5,884
Cyclophosphamide	#4: Cyclophosphamide [Title/Abstract] OR "Cyclophosphamide Anhydrous" [Title/Abstract] OR "Cyclophosphamide, (R)-Isomer" [Title/Abstract] OR "Cyclophosphamide, (S)-Isomer" [Title/Abstract] OR "Cytophosphane" [Title/Abstract] OR "Cyclophosphamide Monohydrate" [Title/Abstract] OR "Cytophosphan" [Title/Abstract] OR "Cytoxan" [Title/Abstract] OR "Endoxan" [Title/Abstract]	54,244
#1 AND #2 AND #3 ABD #3	("Chronic B-Cell Leukemias"[All Fields] OR "b lymphocytic leukemia chronic"[Title/Abstract] OR "Chronic B-Lymphocytic Leukemias"[Title/Abstract] OR "Diffuse Well Differentiated Lymphocytic Lymphoma"[Title/Abstract] OR "Chronic Lymphatic Leukemia"[Title/Abstract] OR "Chronic Lymphatic Leukemias"[Title/Abstract] OR "leukemia chronic lymphocytic"[Title/Abstract] OR "leukemia lymphocytic chronic"[Title/Abstract] OR "CLL"[Title/Abstract]) AND ("Fludarabine"[Title/Abstract] OR "9 beta-D-arabinofuranosyl-2-fluoroadenine monophosphate"[Title/Abstract] OR "fludarabine monophosphate"[Title/Abstract]) AND ("Cyclophosphamide"[Title/Abstract] OR "Cyclophosphamide Anhydrous"[Title/Abstract] OR "Cytophosphane"[Title/Abstract] OR "Cyclophosphamide Monohydrate"[Title/Abstract] OR "Cytophosphan"[Title/Abstract] OR "Cytoxan"[Title/Abstract] OR "Endoxan"[Title/Abstract]) AND ("Rituximab"[Title/Abstract] OR "cd20 antibody rituximab"[Title/Abstract] OR "Rituximab CD20 Antibody"[Title/Abstract] OR "Mabthera"[Title/Abstract] OR "Rituxan"[Title/Abstract]) Filters: Randomized Controlled Trial, Humans, English	28

Searching strategy for Cochrane central registry for clinical trial

Both keywords and medical subject heading were used to search the Cochrane central registry for clinical trials. Four keywords were utilized: Chronic lymphocytic leukemia, Fludarabine, Rituximab, Cyclophosphamide. Then, a limiter "clinical trial" was used to further focus the search. Finally, total 216 results were collected when the search was conducted on 17th November 2022.

Table 2: Search strategy for Cochrane central registry for clinical trials

Keyword or MeSH		Search result
Chronic Lymphocytic leukemia	#1: Chronic Lymphocytic leukemia	1937
Fludarabine	#2: Fludarabine	1617
Rituximab	#3: Rituximab	5694
Cyclophosphamide	#4: Cyclophosphamide	13173
	#1 and #2 and #3 and #4 with limiter: clinical trials	216

Search strategy for Cumulative Index of Nursing and allied health literature (CINAHL)

The abstraction of every article in the CINAHL database was searched by combining four keywords with the Boolean operator "AND": Chronic lymphocytic leukemia, Rituximab, Fludarabine, Cyclophosphamide. Then the search result was

further modified by using the filter "clinical trial". This search was conducted on 17th November 2022, and the number of search results were 94 (Table 3).

Table 3: Search strategy for Cumulative Index of Nursing and allied health literature (CINAHL)

Keywords or MeSH	Search string	Search result
Chronic lymphocytic leukemia Rituximab Fludarabine Cyclophosphamide	AB (chronic lymphocytic leukemia or cll) AND AB (rituximab or anti-cd20 or monoclonal antibody or rituxan) AND AB (fludarabine or fludara) AND AB cyclophosphamide	94

Inclusion criteria

Primary research articles reporting clinical trials, which enrolled chronic lymphocytic leukemia (CLL) patients. Furthermore, these clinical trials should follow good and safe clinical practice guideline advised by Helsinki declaration [25]. Furthermore, studies included in this systematic review were published in peer-reviewed academic journals of English language. Moreover, these articles had compared the efficacy and safety of Fludarabine, Cyclophosphamide, Rituximab to chemotherapy alone in CLL patients. Eligible studies used the diagnostic criteria settled by the international workshop organized on Chronic Lymphocytic leukemia [26]. Studies included in this review were reported overall survival (OS), progression free survival (PFS), objective response rate (ORR), serious adverse effects (SAE). Serious adverse effects should be as described as grade 3 or grade 4 by Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0) [27].

Exclusion criteria

All review articles, systematic reviews with or without meta-analysis were excluded from this systematic study. No *in vivo* animal study or human cell line studies were included in this systematic review. No retrospective or cohort studies were included in this review. No single arm studies were included in this systematic review. Any study which provided any additional treatment in the experimental arm or control arm was excluded. Any study which had not reported outcome of interest were excluded from this systematic review in the screening stage.

Reviewing process

Citations from PubMed, CINAHL, and Cochrane central registry for clinical trials will be downloaded and stored in separate group of the EndNote citation managing software [28]. Then, these groups are amassed in a single group and searched for the duplicates using the EndNote software and duplicates were excluded from the citation utilizing the de-duplication feature of the citation software [29]. Rest of the articles underwent screening based on the reading of the title and abstract sections of the selected articles, and irrelevant articles were excluded from the review. Remaining articles were selected for retrieval; however, some articles could not be retrieved due to several reasons, and these articles were removed from the review. Still remaining studies were scrutinized by reading the full text with special attention to the methodology and result sections. Several articles were excluded based on the predetermined inclusion and exclusion criteria of the review. Rest of the articles were included in the final review.

Outcomes

Primary outcomes of this systematic review are progression free survival (PFS), overall survival (OS), objective response rate (ORR). Secondary outcome is serious adverse effect (grade 3-4 adverse events). PFS is counted from the time of randomization to time of progression of disease either clinically or diagnostically. Median PFS means when at least 50% of the participants progressed on disease. OS is measured from the time of randomization to time of death. ORR is determined by ratio of patient who had any type of response (complete response, partial response, stable disease) and the total number of patients. Safety outcomes are defined by serious adverse effects which is counted as per the definition of common terminology for adverse events (CTCAE) (version 3.0) [27].

Data extraction

A data collection form was generated using the Excel software to extract the background and outcome data from the selected studies [30]. Data was captured for background characteristics of the study, for example, trial name, publication year, journal of publication, registration code of NCT, follow-up duration, and study design etc. Data regarding the patient characteristics were also collected, for example, median age, patient characteristics, inclusion criteria, sample sized in each arm, treatment provided in the experimental arm, and treatment provided in the control arm etc. Regarding the outcome of the included studies following data were collected: median OS, median PFS, ORR, serious adverse effects (grade 3-4 adverse events).

Assessment of the risk of bias

To assess the quality of the included study JADAD score was calculated based on the three primary factors: randomization, blinding, attrition [31]. JADAD score basically a five point score, where one point is for mentioning the randomization while another extra point is given for detailed description of the randomization process which have to internationally accepted [32]. Furthermore, one point is for single blinding where patients do not know the treatment they are receiving, to decrease the bias, however, another point is for double-blinding, which means that both the study participants and study administrators including physician were not sure about the allocation of each student [33]. Finally, if more than 20% patients were lost to the follow-up, then the article receives 0 point, however, if less than one fifth of the patients were lost to the follow-up then the study receives 1 point [34]. These information regarding the quality of the study was collected from the methodology section of each article and for comparison Cochrane collaboration Handbook for systemic reviews on intervention was consulted [35].

Results

Figure 2 shows the algorithm utilized for the present study. PubMed database searching obtained 28 citations, CINAHL database searching provided 94 citations, and Cochrane registry for clinical trials provided 216: in total 338 citations.

From these 338 citations, 29 were found duplicate and removed. Remaining 309 citations underwent screening of Title and Abstracts, of which 267 were found irrelevant to the current systematic review and excluded from the study. Remaining 42 potentially eligible studies were tried to retrieve, however, only 18 could be retrieved. These 18 studies were evaluated for the eligibility based on the inclusion and exclusion criteria of this review, which was previously determined. Four articles were excluded as these were review study, three articles were excluded for being conducted on animals, four articles were removed as these were case studies, and two articles were excluded as these were single arm clinical trials.

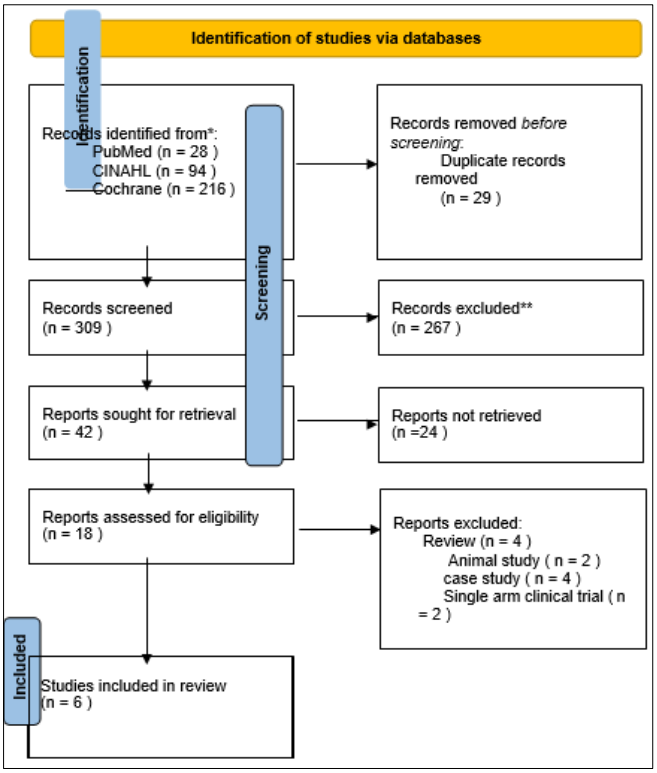

Fig 2: PRISMA flow chart depicting the detailed workflow of this systematic review as records were collected from three different databases, followed by duplicate removal in the identification stage, followed by Title/Abstract screening, retrieval and full text screening. This was followed by eligibility screening based on the inclusion and exclusion criteria [24]

Background attributes of the included studies

Five of the six included studies were randomized, and one was retrospective study [26, 36-39]. One study was conducted in Germany and rest of the studies were conducted in multiple countries [26, 36-39]. Total sample size was 3433, with 1723 patients in the experimental arm, receiving FCR chemoimmunotherapy and 1710 patients were in the control arm, receiving FC chemotherapy alone. Sample size ranges from 817 to 183 [26, 39]. All of these clinical trials enrolled CLL patients, however, from enrolled treatment naïve CLL patients, one trial enrolled recurrent or relapsed CLL patients and another trial enrolled previously treated CLL patients [26, 36-39]. All these clinical trials administered chemoimmunotherapy (FCR) in the experimental arm while chemotherapy alone (FC) was given in the control arm [26, 36-39]. Median age of the enrolled patients ranges from 57 to 62 years [26, 40]. Follow- up duration was varied with Herling *et al.*, (2020) reported the longest follow-up with 55.6 month while Robak *et al.*, (2010) reported the shortest follow-up with only 25 months [39, 40].

Table 4: Basic characteristics of the included studies

Author and date	Study design	Country	Number of Subjects		Patients Characteristics	treatment	Control	Age	Follow up (months)
			E	C					
Wierda *et al.*, 2006 [36]	Retrospective study	11 countries	143	111	Recurrent or relapsed CLL	FCR	FC	62	32
Hallek *et al.*, 2010 [41]	Open label RCT	12 countries	409	408	Treatment naïve CLL patients	FCR	FC	57	48
Robak *et al.*, 2010 [40]	Phase III, RCT	18 countries	276	276	Previously treated CLL patients	FCR	FC	63	25
Molica, 2011 [37]	Open label RCT	12 countries	403	407	Treatment naïve CLL patients	FCR	FC	60	48
Fischer *et al.*, 2016 [38]	Open label RCT	11 countries	410	407	Treatment naïve CLL patients	FCR	FC	61	71
Herling *et al.*, 2020 [39]	Phase III, RCT	Germany, France	82	101	Treatment naïve CLL patients	FCR	FC	58	55.6

Abbreviation: E: Experimental ram, C: Control arm, CLL: Chronic lymphocytic leukemia, FCR: Fludarabine, Cyclophosphamide, Rituximab, FC: Fludarabine, Cyclophosphamide, RCT: Randomized controlled trial

Efficacy outcomes

Median progression free survival (PFS)

All six clinical trials reported median PFS as primary outcome, out of these six clinical trials five reported higher median PFS in the experimental arm, who received chemoimmunotherapy with FCR protocol, compared to the control arm [36-41]. The longest median PFS was reported by Fischer *et al*., (2016) with 56.8 months and the shortest median PFS was reported by Wierda *et al*. with 32 months [36, 38]. The highest PFS benefit was reported by Fischer *et al*. with 23.9 months (56.8 vs 32.9 months) [38].

Median overall survival (OS)

Out of the six clinical trials included in the systematic review, four clinical trials reported median OS as a primary outcome [36, 38, 39, 41]. The median OS so high that in two trials median OS did not reach even after 86 and 77 months, which means that less than 50% patient did not die in these two clinical trials [38, 39]. However, lowest median OS was reported by both Wierda *et al* (2006) and Hallek *et al*., (2010) with both at 49 months [36, 41].

Objective response rate (ORR)

Five of the six clinical trials reported ORR as primary outcome, out of which four clinical trials reported higher response rate in the experimental arm, receiving chemoimmunotherapy (FCR) compared to the control arm, receiving chemotherapy alone (FC) [36-39, 41]. The highest ORR was reported by Herling *et al*., (2020) with 92.7%, however, the lowest ORR was reported by Weirda *et al*., (2006) with only 50.35% response rate [36, 39]. Furthermore, the ORR benefit was reported by Herling *et al*., (2020) with 23.2% increased ORR in the experimental arm [39].

Grade 3-4 adverse effects

All six of these clinical trials reported grade 3-4 adverse effects and all six of these trials reported higher adverse effects in the experimental arm, receiving chemoimmunotherapy (FCR), compared to control arm participants, receiving chemotherapy alone (FC) [36-41]. The highest increase in serious adverse effects was reported by Fischer *et al*., (2016) with 20% (55% versus 35%) increase in serious adverse events [38].

Table 5: Outcomes of the included studies

Author and date	Median PFS (months)		Median OS (months)		ORR		Grade 3-4 adverse effects		JADAD score
	E	C	E	C	E	C	E	C	
Wierda *et al*., 2006 [36]	32	36	49	31	50.35%	60.36%	66%	47%	2/5
Hallek *et al*., 2010 [41]	48	32	49	33	55%	45%	34%	21%	4/5
Robak *et al*., 2010 [40]	30.6	20.6	N R	N R	N R	N R	38%	34%	3/5
Molica, 2011 [37]	51.8	32.8	N R	N R	86%	81%	45%	35%	4/5
Fischer *et al*., 2016 [38]	56.8	32.9	Not reached	86.0	87%	83%	55%	35%	4/5
Herling *et al*., 2020 [39]	54	39	Not reached	77.0	92.7%	69.5%	61.0%	41.5%	5/5

Abbreviation: PFS: progression free survival, OS: overall survival, NR: Not reported

JADAD score

Five out the six clinical trials were calculated to have 3 or more score in JADAD scale [31] (Table 6). One phase III RCT scored 5 out of 5 and three RCT scored 4 out of 5 [37-39, 41] (Table 6). Only retrospective study scored 2 out of 5 in JADAD scale due to lack of blinding and improper randomization [36]. Four clinical trials suffered from attrition bias as more than 20% patients were lost to the follow-up [37, 38, 40, 41] (Table 6). Only one trial did not perform randomization as this was a retrospective clinical trial [36]. Two trials failed on the double blinding which is the source of observer bias [36, 40] (Table 6).

Table 6: Calculation and breakdown of JADAD score of the included studies

Author and date	Randomization	Appropriateness of randomization	Allocation concealment	Double blinding	Attrition (more than 20%)	Total Score
Wierda *et al*., 2006 [36]	0	0	1	0	1	2/5
Hallek *et al*., 2010 [41]	1	1	1	1	0	4/5
Robak *et al*., 2010 [40]	1	1	1	0	0	3/5
Molica, 2011 [37]	1	1	1	1	0	4/5
Fischer *et al*., 2016 [38]	1	1	1	1	0	4/5
Herling *et al*., 2020 [39]	1	1	1	1	1	5/5

Discussion

This systematic review evaluating the efficacy and safety of chemoimmunotherapy FCR compared to FC chemotherapy alone in patients with CLL produced several key findings. This systematic review consistently reports that chemoimmunotherapy with FCR is superior to chemotherapy alone regimen regarding the OS and PFS, however, FCR also causes higher toxicity in CLL patients.

Five out of six clinical trials reported higher PFS in chemoimmunotherapy FCR recipient CLL patients compared to FC chemotherapy alone recipients CLL patients [37-41]. This superiority in PFS possibly originated from the additional treatment of Rituximab as Rituximab kills leukemia cells in three different mechanisms: direct lysis, complement-mediated cell death, and antibody-mediated cell cytotoxicity (ADCC) [9]. Furthermore, Fischer *et al*., (2016) reported the highest PFS (56.8 months) along with the highest PFS benefit of 23.9 months, which possible originated from the fact that this phase III randomized controlled trial enrolled patients who were previously untreated, furthermore, this trial also reported hazard ratio for PFS of 0.47, which means 53% improvement in PFS compared to FC chemotherapy alone, which was clinical significant [38].

Four out of six clinical trials reported OS and all of these reported higher OS in the experimental arm. In two trials OS was so high that median OS was not reached after 86 and 77 months, meaning that less than half the total patients (50% patients) died in the clinical trial, however, Fischer *et al*.,

(2016) reported hazard ratio as 0.62, which expressed 38% increase in the OS [38, 39]. This longevity in OS might have originated from the addition of the Rituximab as this drug potentiates human immune system against the CLL cells, leading to the long-standing action against the cancer cells, which is reported as long remission in CLL patients [10]. Pre-clinical studies also reported synergistic action of Rituximab in combination with FC chemotherapeutic regimen, showing that each type of therapy help another one.

Four out of five clinical trials reported higher response rate in the experimental arm receiving chemoimmunotherapy compared to the control arm receiving FC chemotherapy alone [37-39, 41]. More importantly, majority of the response in the FCR arm is complete response while majority of the response in FC arm is either partial response or stable disease, which is inferior to the complete response [37-39, 41]. This higher rate of response is also consistent with other clinical trials involving multiple types of drugs, for example, CLL10 administered Bendamustine along with Rituximab also received similar high response rates [42]. Human cell line-based studies showed that this added benefit originated from the multiple mechanisms of action from different groups of drugs as Rituximab kills cells by ADCC and NK cells, whereas FC chemotherapy kills any dividing cells non-specifically by cross-linking DNA and RNA along with inhibiting protein synthesis [43]. When all these mechanisms work separately, they also help killing by other mechanism, for example, a cell injured by chemotherapeutic agents become more immunogenic due to the stress created in the surrounding tissue, which was reported in human colon cancer cells [44].

All six clinical trials reported higher adverse effects among FCR recipient CLL patients compared to FC chemotherapy alone recipient CLL patients [36, 38- 41]. Most common of these adverse effects were Leukopenia, neutropenia, causing bacterial, viral infections as well as increasing the possibility of opportunistic infections [36, 38-41]. More importantly, a good proportion of patients developed second malignancy (both solid tumours and myelodysplasia), for example, Wierda *et al*., reported that 23 out of 143 patients developed second malignancy over the next 10 years [36]. However, no studies conducted any exploratory analysis between the patients developing secondary malignancy and the patients no developing secondary malignancies [36-41]. Higher rates of adverse effects among the FCR recipients is explained by different adverse effects based on the different mechanisms of action, for example, adverse effects of FC chemotherapy added to the adverse effects of Rituximab, creating a higher rates and diverse types of grade 3-4 adverse events [45].

Majority of these trials of good quality as four trials scored 4 or more (out of 5) of in the JADAD scale [37-39, 41]. However, Wierda *et al*., score lowest (2/5) as this was a retrospective trial, leading to higher possibilities of bias [36]. Majority of the trials suffered from attrition as more than 20% lost to follow-up possible due the long follow-up and occasionally asymptomatic nature of the disease [37, 38, 40, 41]. Furthermore, two trials suffered from observer bias due to lack of double blinding of the clinical trials [37, 38]. One trial did not mention the randomization process with enough detail in the methodology [36].

None of the studies conducted or reported a cost-effectiveness analysis, however, a separate cost-effectiveness analysis was reported in the USA for FCR chemoimmunotherapy treatment [46]. However, none of the studies discussed the potential ways of integrating this novel treatment into the health system, however, the drug was introduced National comprehensive cancer network (NCCN) guideline, which indicates that this is possible to integrate in any treatment protocol [47].

Previous systematic review done on this topic did include different treatment regimens in the control arm, making the comparison less focused [48]. Even more, this systematic review included studies on both treatment naïve and recurrent CLL patients, leading to the ill-defined result [48].

Limitations

This systematic review was conducted by a single scientist; however, every systematic review should be conducted by at least two scientists. This systematic review does not include any quantitative analysis, which limited its purpose in presenting quantitative results. Only six articles were included in this review as the more published studies could not be found. Only three databases were searched due to time and resource constraints. Out of 42 articles, 24 articles could not be retrieved due to lack of subscriptions or funding. None of the studies reported cost-effectiveness analysis, so a comparison on cost-effectiveness could not be made.

Conclusion

This systematic review showed that chemoimmunotherapy with FCR regimen has higher PFS compared to FC chemotherapy alone in CLL patients. Furthermore, this review also reported higher OS in FCR recipient CLL patients compared to FC recipient CLL patients. Moreover, this systematic review reported higher ORR in FCR receiving experimental arm. However, FCR recipients suffered from higher adverse effects along with higher number of second malignancy compared to chemotherapy alone recipients. In future trial should be conducted with larger sample size to get more data regarding the rare side effects and variety of second malignancy. More importantly, trial should be conducted with sub-group analysis between the prognostically important IGHV mutations, 17p and 11q deletions. There should be additional sub-group analysis between the fit and unfit patients with poor performance status. These future trials should also collect separate data on adverse effects and second malignancy in patient over 65 years and under 65 years of age. Future clinical trials should perform cost-effectiveness analysis with a detailed discussion on the integration of this new treatment in the treatment paradigm. There should be clinical trials in the future with the combination of emerging treatments like ibrutinib or Alemtuzumab to find out the optimum treatment with the reporting of quality of life as an outcome.

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