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Donor origin CAR T cells: graft versus malignancy effect without GVHD, a systematic review

CD19, CD20 chimeric antigen receptor T (CAR T) cell therapy has shown promising results for the treatment of relapsed or refractory hematological malignancies. Best results have been reported in acute lymphoblastic leukemia patients with a complete response rate above 80%. Patients who received donor-derived CAR T cells for the relapsed malignancy after stem cell transplantation (allogeneic hematopoietic stem cell transplant) were identified from the published trials. A total of 72 patients from seven studies were treated with donor-derived CAR T cells. Only five out of 72 patients (6.9%) developed graft versus host disease. Use of donor-derived CAR T cell for relapse prophylaxis, minimal residual disease clearance or salvage from relapse is therefore highly effective, and risk of graft versus host disease flare is very low. Side effects include cytokine release syndrome, tumor lysis syndrome, B-cell aplasia along with CNS toxicity.

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Allogeneic hematopoietic stem cell transplant (Allo-HSCT) is routinely used for treatment of aggressive hematological malignancies. Currently it is only curative option available for relapsed acute lymphoblastic leukemia (ALL) with long-term survival and cure rate in the range of 25–65% [1]. Relapse following an allogeneic hematopoietic stem cell transplant remains a major challenge in the treatment of hematologic malignancies. The median survival for adult ALL in this setting is less than 6 months. Post-transplant relapse survival rates at 1, 2 and 5 years are estimated to be around 30, 15 and 10%, respectively [2]. Treatment options such as donor lymphocyte infusion (DLI) and blinatumomab have shown limited success in the setting of relapse. Spyridonidis *et al.* reported DLI is used (with or without pre DLI chemotherapy) in approximately 20% of ALL patients to treat post Allo-HSCT relapse

with variable success [3]. Rationale for the use of DLI is based on graft versus leukemia augmentation but this effect is very in cases of ALL [4]. The use of DLI is limited by a mere increase in median survival by 6 months and a significant risk (40–60%) of acute and chronic graft-versus-host disease (GVHD) and additional risk of marrow aplasia [5,6].

The Bi-specific T-cell engager blinatumomab can engage donor T cells in setting of B ALL relapse disease [7]. Single-agent blinatumomab in the setting of relapsed or refractory ALL has shown to achieve a complete response (CR) rate of 70% with median overall survival of 9.8 months [8]. It is an effective option for salvage and it is used as a bridge to Allo-HSCT after achieving CR, however it is not curative in many cases [7].

Treatment with CD19 chimeric antigen receptor T (CAR T) cells therapy is a novel method of targeted immunotherapy for the

Faiz Anwer^{*,1,2}, Al-Aman Shaukat^{*,3}, Umar Zahid^{4,5}, Muhammad Husnain⁴, Ali McBride⁶, Daniel Persky^{1,2}, Melissa Lim², Nida Hasan⁷ & Irbaz Bin Riaz⁴

¹Department of Medicine, Hematology & Oncology, University of Arizona, Tucson, AZ, USA

²Arizona Cancer Center, University of Arizona, Tucson, AZ, USA

³James Paget University Hospital, Norfolk, UK

⁴Department of Medicine, University of Arizona, Tucson, AZ, USA

⁵College of Public Health, University of Arizona, Tucson, AZ, USA

⁶College of Pharmacy, University of Arizona, Tucson, AZ, USA

⁷University of Arizona Zuckerman College of Public Health, Tucson, AZ, USA

*Author for correspondence:

Tel.: +1 520 626 3191

Fax: +1 520 626 8944

anwerf@email.arizona.edu

^{*}Authors contributed equally

treatment of B-cell mediated hematological malignancies such as B-cell ALL, B-cell chronic lymphocytic leukemia (CLL) and CD 19 expressing B-cell non-Hodgkin lymphoma [9–12]. Donor origin CAR T-cell-based therapy has the potential to combine the graft versus leukemia effect (augmented with DLI use), with cell mediated targeted elimination of CD19 expressing B cells (seen with use of bispecific antibody such as blinatumomab). Composition of DLI is different from CAR T cells and predominantly there are donor peripheral blood mononuclear CD3⁺ cells, B cells, dendritic cells and other cell types [13]. There is extensive variability in the processes for CAR T production, effector T-cell phenotypes, costimulatory domains and other associated factors that need to be considered before drawing inferences from results summarized in this manuscript. There is literature to support that blinatumomab-refractory B cell disease is responsive to CAR T treatment [14]. We present a literature review to identify the current evidence for efficacy and safety of donor-derived CAR T cells after failure of an Allo-HSCT and whether or not there is associated high risk of GVHD flare with this treatment. Readers should keep in mind that most of the patients who received donor-derived CAR T cells were excluded if they had significant history of acute or chronic GVHD.

Materials & methods

The meta-analysis was designed in accordance with the principles set by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist.

Eligibility criteria

Inclusion criteria specified all clinical studies with adult patients who had B cell malignancy (ALL, CLL and Non-Hodgkin lymphoma) and underwent anti-CD 19 or anti-CD20 CAR T-cell therapy. Later we extracted studies for analysis with data on donor-derived CAR T cells.

Exclusion criteria

We excluded ongoing clinical trials without reported outcomes, studies not reporting survival outcomes and studies with other than anti-CD19 or anti-CD20 CAR T cells.

Search strategy

Literature search was performed using following electronic bibliographic databases: MEDLINE (Ovid SP and PubMed), EMBASE, The Cochrane Library (Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Trials, Scopus and Web of Science). The initial search was not restricted to English. The searches were repeated just

before the final analyses and further studies retrieved for inclusion till 25 May 2016. The bibliographies of retrieved articles and previous review articles were hand searched to obtain additional articles. Kebriaei *et al.* study was added after May 2016 literature search to include data about additional 19 patients where donor CAR T cells were used for prophylaxis.

Data extraction

Using the search strategy, we obtained titles and/or abstracts of retrieved studies and imported them to endnote. Two investigators (Umar Zahid, Muhammad Husnain) independently screened the titles and abstracts; the full texts were screened if the articles met the inclusion criteria. Full text of these selected articles were obtained and evaluated by two investigators (Faiz Anwer, Irbaz Bin Riaz) to confirm eligibility for inclusion. Data were extracted using a structured template and disagreement resolved with consensus during the process of screening and data extraction. A standardized data extraction form was used to extract the following fields: Author, year of publication, phase of clinical trials, total number of patients in the study, number of post-HSCT patients who received donor-derived CAR T cell, chimeric T cell construct, trial criteria for CAR T use, preconditioning, lymphodepleting therapy use, type of B malignancy, type of CAR T, source of T cells, toxicity, cytokine release syndrome (CRS), neurotoxicity, range of cell dose used and CD 19 efficacy and complication with flare of GVHD.

Results

A total of seven eligible articles were identified for inclusion in the final review [14–20]. A total of 72 patients were treated with donor-derived CAR T cells in these studies. There were 50 patients with ALL, nine patients with CLL and 13 patients with non-Hodgkin lymphoma. Due to dose escalation trial design of many studies, heterogeneity of patient population and various settings (prophylaxis vs salvage) in which CAR T cells were used, it was not possible to summarize disease response about individual patients or subset of disease types. The longest duration of CR reported in the trial was 30 months in a patient of CLL [21]. Only five out of 72 patients developed GVHD (6.9%). Results of individual trials with detail are summarized in detail in Table 1.

Discussion

Published data from multiple early phase clinical trials on the efficacy of adoptive immunotherapy using CD 19 and CD 20 CAR T are very promising. There are significant differences in response rates according to disease subtype. Review of data from Phase I and

Table 1. donor-derived chimeric antigen receptor T-cell therapy in post hematopoietic stem cell transplant relapse patients with B cell malignancy.

Study (year), phase of study	Total patients (n)	Number of post HSCT patients given donor-derived CAR T cell/T-cell construct	Trial criteria for CAR T use/preconditioning use and type	Type of B malignancy	Type or source of donor CAR T	Toxicity: (CRS/ neurotoxicity)	Cell dose	Efficacy	GVHD
Cruz <i>et al.</i> (2013), Phase I	8	8/donor-derived VSTs (CMV, EBV, and Adv-specific) CD19-specific CAR	B malignancy, Post-allo HSCT relapse, high risk of relapse after HSCT/No preconditioning	ALL = 4 & CLL = 4	Donor-derived-CD19-VST/Donor source Peripheral blood mononuclear cells with CD28 costimulatory endodomain	NO CRS/no neurotoxicity	Dose escalation $1.5 \times 10^7/\text{m}^2$, $4.5 \times 10^7/\text{m}^2$, $1.2 \times 10^8/\text{m}^2$ total cell numbers (not D19.CAR+ cells 0	CR-1/6, PR-1/6, SD-1/6, PD-3/6, 2 high-risk patients remained in CR	No GVHD
Kochenderfer <i>et al.</i> (2013), Phase 1	10	10/anti-CD19-CAR T cells	Post Allo SCT residual disease/no preconditioning used	CLL = 4 MCL = 4 DLBL = 2 CD19 +ive B-cell malignancy persisted despite alloHSCT and standard DLI	Donor-derived-CD19/Allo SCT donor	Grade 3 toxicity CRS = 4 patients TLS = 1 patients No other patients had grade 3 toxicity	0.4×10^6 – $7.8 \times 10^6/\text{kg}$	CR = 1, PR = 1, SD = 6, PD = 2	No GVHD
Davila <i>et al.</i> (2014)/Phase 1	16	4/CD 19–28z T cells	Refractory or relapsed B-ALL/Cyclophosphamide (1.5 to $3.0 \text{ g}/\text{m}^2$)	B-ALL = 16	4/4 Donor-derived 19–28z CAR T cells	Neutropenia 12/16, hypotension 6/16, chills 1/16,	3×10^6 CAR T cells/kg	Overall CR = 88% Patients successfully proceed to receive Allo SCT 44%	NO GVHD
Maude <i>et al.</i> (2014), Phase I/IIA Study	30	18/CD19-BB zeta Transgene (CTL019)	Relapse/primary refractory 29 B-cell ALL, 1 patient relapsed T-cell ALL CD19 +ive/yes variable preconditioning	B-ALL 29 T ALL 1	Autologous T Cells/median donor chimerism at the time of leukapheresis was 100% (range: 68–100)	All patients CRS/13 CNS toxicity/B Aplasia in all patients	A split-dose strategy dose of 0.76 to 20.6×10^6 CTL019 cells/kg	Overall CR = 90% At 6 months, EFS 67% OS 78%. Similar out comes for post Allo SCT relapse pt	No GVHD

ALL: Acute lymphoblastic leukemia; CAR T: Chimeric antigen receptor T cell; CLL: Chronic lymphocytic leukemia; CMV: Cytomegalovirus; CR: Complete response; CRS: Cytokine release syndrome; DLBL: Diffuse large B-cell lymphoma; EBV: Epstein–Barr virus; GVHD: Graft versus host disease; HSCT: Hematopoietic stem cell transplantation; MCL: Mantle cell lymphoma; NHL: Non-Hodgkin lymphoma; PD: Progressive disease; PR: Partial response; SD: Stable disease; TLS: Tumor lysis syndrome; VST: Virus-specific T cell.

Table 1. donor-derived chimeric antigen receptor T-cell therapy in post hematopoietic stem cell transplant relapse patients with B cell malignancy (cont.).

Study (year), phase of study	Total patients (n)	Number of post HSCT patients given donor-derived CAR T cell/T-cell construct	Trial criteria for CAR T use/ preconditioning use and type	Type of B malignancy	Type or source of donor CAR T	Toxicity: (CRS/ neurotoxicity)	Cell dose	Efficacy	GVHD
Dai <i>et al.</i> (2015)/Pilot trial	9	3/Mixture of CART-19 CD8+ive Cells 70.87%, CD62L+ 63.36%, central memory T cell phenotype 15.54% +ive	Refractory B-ALL/ only 2 received Conditioning with C-MOAD	ALL = 9	Donor-derived CD19-CAR T cells/ only 2 received donor-derived T cells	CRS 4/9 (fever 4/4, dyspnea 2/4, capillary leak syndrome 1/4, oliguria 2/4), dyspnea 2/4	$3.0 \times 10^6 - 7.3 \times 10^6$ /Kg CART CD19 +ive cells	2/2 Response 100% CR = 1, PR = 1	Grade 2–3 GVHD in 2 patients
Brudno <i>et al.</i> (2016)/Phase 1	20/first 10 patients data published in Kochenderfer 2013 and excluded	10/murine single-chain variable fragment Ag recognition domain, a CD28 costimulatory domain, and a CD3z T-cell activation domain	CD19 B malignancy refractory to allo-HSCT/no preconditioning used	CLL = 1, MCL = 1, ALL = 4 and DLBCL = 4	Donor-derived CD19-targeted CAR T cells	Grade 3 CRS 8 patients, CNS toxicity	$3.1 \times 10^6 - 8.2 \times 10^6$ /kg anti-CD19 CAR T cells	CR = 5, PR = 1, SD = 2, PD = 2	NO GVHD
Kebrl <i>et al.</i> (2016)/Phase 1	26	19/DNA plasmids from the SB platform Second-gen	<i>Sleeping Beauty</i> mediated CD19-specific CAR T cells, prophylaxis, No prior GVHD/no preconditioning	B-ALL = 17 B NHL = 02	CD19-targeted/ donor-derived	NO	$1 \times 10^6 - 5 \times 10^8$ /kg ant CD19	After Allo SCT 12-month PFS 53% and OS 63%	GVHD 2 acute, one chronic

ALL: Acute lymphoblastic leukemia; CAR T: Chimeric antigen receptor T cell; CLL: Chronic lymphocytic leukemia; CMV: Cytomegalovirus; CR: Complete response; CRS: Cytokine release syndrome; DLBL: Diffuse large B-cell lymphoma; EBV: Epstein-Barr virus; GVHD: Graft versus host disease; HSCT: Hematopoietic stem cell transplantation; MCL: Mantle cell lymphoma; NHL: Non-Hodgkin lymphoma; PD: Progressive disease; PR: Partial response; SD: Stable disease; TLS: Tumor lysis syndrome; VST: Virus-specific T cell.

Phase II studies [22] on efficacy of CAR T cells for hematological malignancies, reveal higher response for ALL (80%) and relatively lower response for CLL and B non-Hodgkin lymphoma (50%) [23,24]. The circulating pool of CD 19 targeted immunity CAR T cells persist for longer duration, and achieve durable remission. Treatment-related complications are due to so-called ‘on-target’ and ‘on-target/off-tumor’ effects, such as cytokine releases syndrome, tumor lysis syndrome, long term B-cell aplasia, hypo-gammaglobulinemia and a spectrum of CNS-related side effects [21].

Post Allo-HSCT relapses data from pediatric and adult trials show dismal prognosis, even patients undergoing a second transplant have poor prognosis [25]. Current guidelines for post-transplant relapse management are lacking and there is little consensus on treatment. Treatment is variable and mainly based on individual parameters such as patient factors (age, comorbidity, active infections, donor availability), disease related factors (remission status, potential targets for therapy), available salvage options and clinical trial availability. Other than palliative care for very sick patients after ALL relapse, transplant physicians routinely explore the possibility of a second Allo-HSCT after salvage with reduction in immune suppression, multi-agent chemotherapy and occasionally DLI [26].

Alternative therapeutic approaches may include novel immune-therapies including blinatumomab and CAR T cells. Kalos and colleagues treated three advanced stage CLL patients with CAR T cells in a Phase I trial in Pennsylvania and all three patients remained in remission 4.5 years after therapy [27]. Maude *et al.* treated thirty transplant refractory ALL with CD19 CAR T cells and showed a complete remission in 90% of the patients, provided evidence of efficacy in bispecific antibody blinatumomab refractory cases as well as successful salvage for post allo-HSCT relapse in more than 80% of cases [14]. blinatumomab induced donor T-cell activation for post stem cell transplant-relapsed ALL has been well described [7], but similar data for donor origin CAR T are limited and emerging. Two recent reviews on the subject summarize the initial gains and challenges to the approach [28,29]. Donor origin CAR T cells can be generated by collecting effector cells from original allogeneic donor (Allo-CAR T) or collected from HCST recipient but belong to original allogeneic donor immune system (Allo-auto CAR T). Phenotypically and genetically Allo-CAR T and Allo-auto CAR T are expected to be identical in nature and in anti-tumor actions. CAR T cells recognize target antigens in a non-HLA dependent manner. In this systematic review our data on 72 patients treated with donor-derived CAR T cells after HSCT failure, published in seven clinical trials, provide a proof for

efficacy and safety for this approach. Most of the studies summarized in our review have limited number of patients, are either Phase I or pilot studies and with few studies with dose escalation design, it is not possible to draw conclusions or perform statistical tests for efficacy and at best we could summarize data in a simple descriptive manner. These studies are mainly exploring primary objectives about safety, feasibility, T-cell persistence and secondary objectives on therapy efficacy along with side effects along with emergence of GVHD. Cruz *et al.*, Phase I donor-derived-CD19 CAR-modified virus-specific T cells, specific for cytomegalovirus, Epstein–Barr virus and adenovirus should be considered unique and similar studies with emerging data need to be looked closely for similarity and differences when compared with other donor CAR T cells not linked with viral activation. In Cruz *et al.* study [15] two out of six patients with relapsed disease showed objective responses (one complete response and one partial response) and two patients having received CD19 CAR-virus-specific T cell cells while in remission originally, but with high relapse risk, remained in remission without flare of GVHD. Dai *et al.* in their 2015 study [17] looked at the role of donor-derived CAR T cells in two out of nine patients with chemo-refractory B-cell ALL. There was a durable CR in one patient and a partial response in the other patient with overall survival of 8 and 12 weeks, respectively. Both patients had clinically significant GVHD, thus raising the concern for some risk of GVHD associated with donor-derived CAR T therapy. Kebriaei *et al.* study also had very low rate but positive data for GVHD, one case had grade 1 skin, other patient required systemic steroids and third developed liver involvement and later died of liver related issues. Four out of 16 patients in Davila *et al.* study [16] patients who received donor-derived CAR T cells showed CR of 88% for the entire group (n = 16). Brudno *et al.* in 2016 [18] at the NIH reported out of these 20 patients, six achieved complete remission (CR), and partial remission was seen in two patients. The response rate was highest for ALL with four out of five patients obtaining CR. The 6-month event-free survival of all 20 treated patients was 39% and there was no incidence of acute GVHD. Maude *et al.* treated a total of 30 patients, 18 out of 30 had undergone stem-cell transplantation at the time of CAR T cells. In post allo-SCT relapse group CR rates were 83%. Compared with conventional approaches (DLI, reduction in immune suppression, salvage chemotherapy) achievement of this high CR rate without additional GVHD by donor-derived CAR T is remarkably high. In this paper, median donor chimerism at the time of leukapheresis for T-cell collection was 100% (range, 68 to 100), but it is difficult to tell

how many patients had donor origin or recipient origin CAR T [14].

Despite limited number of patients in mainly Phase I dose escalation studies where focus was on safety, many studies demonstrated CAR T cells persisted for longer duration, we did not find any evidence for allogeneic rejection of CAR T cells. More studies with Phase II/III design are required on this subject. Lymphodepleting chemotherapy have shown to enhance CAR T-cell responses by various mechanisms [30] including eradicating regulatory T cells, eliminating competing immune cell but only minority of patients included in this review received any lymphodepleting therapy. As patients included in this review received CAR T after relapse of disease happened after stem cell transplant, theoretically lymphodepleting therapy had the potential to weaken the allogeneic graft. On the other hand it has the potential to improve control of relapse disease and enhance CAR T expansion and hence better disease control. Based on the evolving data authors suggest that allogeneic (donor-derived) CAR T cell therapy is efficacious and safe option for treatment of relapsed B-cell malignancies after failure of Allo-HSCT. Patients with significant GVHD (beyond grade 1) were excluded from these trials and only patients with no or low-grade GVHD received donor-derived CAR T cells. Many responses observed in the included trials were transient. Based on our review findings we hypothesize that patients even with more than grade 1 GVHD can safely receive donor origin CAR T therapy. There are no data to support this theory at this point but likely post transplant relapse patients will get benefit as otherwise there is dismal prognosis for such patients with active GVHD and leukemia relapse. The findings in our review point toward a future when antigen-specific T-cell therapies will play a central role in preemptive, prophylaxis and salvage for aggressive B-cell malignancies.

Conclusion

CAR T cells therapy is targeted immunotherapy, currently in the early stages of development for aggressive hematological diseases such as B-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia and B-cell lymphoma. Adoptive T-cell therapy has shown good responses for relapsed/refractory disease. Highest responses ($\geq 80\%$ complete remission rate) have been reported for acute lymphoblastic leukemia patients followed by responses in chronic lymphocytic leukemia and B-cell non-Hodgkin lymphoma. CAR T cell therapy can effectively salvage patients even after relapse following allogeneic stem cell transplantation. There is concern that donor-derived CAR T cells if used following Allo-HSCT can trigger GVHD. In this systematic review of the literature, we conclude that there is a very low risk of GVHD flare with the use of donor-derived CAR T cells.

Future perspective

Over next 5–10 years, CAR T-based immunotherapy will continue to improve in efficacy and safety and its role will evolve not only for hematological malignancies, but also for solid organ malignancies. CAR T will become routine part of front-line targeted/personalized anticancer therapy with or without concomitant use of other drugs such as cytotoxic agents. Antibodies (naked, bispecific antibody blinatumomab or linked with radio-isotopes or cytotoxic chemicals), immune-modulators, an expanding list of check point inhibitors/stimulators, small molecule inhibitors, will be integrated into treatment paradigm along with gene therapy and highly active CAR T cell therapy. Current challenges to overcome include poor T-cell collection, longer processing time, lack of 'Off-the-shelf' effector lymphocyte product for emergent situations, 'On target/off tumor' effects with CRS, B-cell aplasia, hypogammaglobinemia, infection

Executive summary

- Treatment with chimeric antigen receptor T (CAR T) cells therapy is a novel method of targeted immunotherapy, currently in the early stages of development mainly for acute lymphoblastic leukemia, chronic lymphocytic leukemia and B-cell lymphoma. Adoptive T cell therapy is currently being used for relapsed/refractory disease with favorable responses. Highest responses are seen in acute lymphoblastic leukemia patients ($\geq 80\%$ complete response rate) followed by responses in chronic lymphocytic leukemia and B non-Hodgkin lymphoma. Use of CAR T cell can effectively salvage patients even after relapse following allogeneic stem cell transplantation.
- There are many challenges and serious side effects with the use of CAR T cell therapy, mainly cytokine release syndrome, B-cell aplasia, CNS toxicity and disease relapse. Effective therapies and strategies are under development to minimize toxicity with adoptive T cell therapy.
- There is concern that donor-derived CAR T cell if used for prophylaxis, minimal residue disease clearance, consolidation or salvage from hematological malignancy following Allo-HSCT can trigger graft versus host disease and in this review we found the risk of graft versus host disease flare in this setting is very low and use of adoptive CAR T therapy using donor-derived cells is highly effective. CAR T cell therapy is under extensive testing and will be tested as a part of consolidation therapy or maintenance therapy for relapse risk reduction.

risks (fungal, viral, bacterial), CNS toxicity due to blood brain barrier permeable CAR T and non CAR T-cell recruitment/T-cell hyper activation leading to unchecked cytokine releases in CNS. These issues likely will be resolved by better construct design (high efficacy but 1–2 log lower dose), dual or multitargeting of antigens on tumor surface, cytokine neutralizing agents (like tocilizumab), intracellular pathway inhibition (i.e., Jak inhibitors) for reduction in cytokine release without impairing efficacy and stable cell product to avoid hyper stimulation. Other challenges such as poor cell proliferation, *in vivo* expansion and shorter life span of CAR T cells will be resolved by use of cells with ‘stem cell phenotype-like memory T cell’ and use of immune cell modulators.

Another challenge is disease relapse with ‘target negative biology’ (i.e., CD19⁺ ALL relapse after CD19 CAR T cell therapy). This risk can be reduced by dual or multi-targeting (CD19/CD22 for an example) of tumors using adoptive cells with multiple targets on a single cell (one chimeric receptor vs multiple chimeric receptor expression) or a combination of independently constructed ‘cross-covering’ CAR T cells.

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Author contributions

IB Riaz, U Zahid, A Shaukat, M Husnain, D Persky and F Anwer designed the study. IB Riaz, M Husnain and F Anwer searched for studies for the systematic review. All authors performed the study, contributed to data extraction, analyzed the data and wrote the paper.

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