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# Triple-negative Breast Cancer Therapy Using RNA Nanoparticles Targeting Stem Cell Markers with Anti-miRNA: A Systematic Review and Meta-Analysis

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**ABSTRACT**

**Background and aim:** The present study is the first systematic review and meta-analysis study conducted to investigate the effectiveness of anti-miRNA for triple-negative breast cancer therapy using RNA nanoparticles targeting Stem Cell markers.

**Material and methods:** The Present study is a systematic review and meta-analysis based on PRISMA 2020 Checklist. A search of PubMed, Scopus, Web of Science, EBSCO, ISI Web of Knowledge, and Embase databases was conducted until April 2023 in order to identify systematic literature. The 95% confidence intervals for effect size have been calculated using the fixed effect model and the inverse-variance method, respectively. A meta-analysis was conducted using Stata/MP v.17.

**Results:** An initial review was conducted by eliminating duplicate studies, reviewing 103 abstracts, and reviewing the full text of 11 studies by two authors. Finally, two studies were selected. The average hydrodynamic diameter of 3WJ/CD133apt/anti-miR21 nanoparticles was 10.30 nm (ES, 10.30 nm 95% CI 7.10 nm, 13.51nm) with low heterogeneity (I2=0%; P =0.86). Zeta potential was determined to be -25.19 mV (ES, -25.19 mV 95% CI -39.57 mV, -10.81 mV).

**Conclusions:** 3WJ/CD133apt/anti-miR21 nanoparticles can be considered a suitable option for selecting a Triple-negative breast cancer treatment (TNBC).

## 1. Introduction

Triple-negative breast cancer (TNBC) is a type of breast cancer in which the cancer cells do not have estrogen receptor (ER) or progesterone receptor (PR).[1] Also, unlike other cancers, these types of cancer cells will produce a smaller amount of HER2 proteins. About 15 to 20% of all breast cancer cases are related to TNBC.[2, 3] Hormonal treatment based on trastuzumab is not suitable in patients with TNBCT. Based on the available evidence, this type of cancer metastasizes to the brain, bones, and lungs and is associated with short survival and poor prognosis.[4] Treatment for these patients generally includes pre-operative neoadjuvant chemotherapy; Unfortunately, this treatment cannot cure the disease, and most patients do not recover after chemotherapy. A three-way junction packaging RNA (3WJ-pRNA) can be designed to contain therapeutic agents such as miRNA, chemotherapy drugs, and siRNA.

On the other hand, it contains target ligands such as chemical moieties or RNA aptamers, as well as imaging agents. be included and can maintain folding properties.[5-7] Studies have shown that the chemical stability is improved after the 20-fluoro (20 F) modification on the ribose.[8, 9] No intrinsic toxicity or histological damage was reported for pRNA3WJ in animal studies after systemic injection into a mouse model.[5, 10] The use of multifunctional RNA nanoparticles based on pRNA-3WJ scaffold to deliver antimiR21 has been introduced to realize targeted therapy for TNBC and improve the bioavailability of drugs.[11] CD133 is one of the markers of breast cancer stem cells, which according to the available evidence, is one of the most important markers for diagnosing cancer stem cells and is expressed in patients with TNBC.[12] Considering the importance of the subject and the lack of comprehensive results, the present study is the first systematic review and meta-analysis study conducted to investigate the effectiveness of anti-miRNA for triple-negative breast cancer therapy using RNA nanoparticles targeting Stem Cell markers.

## 2. Material and methods

### *Search strategy*

A search was conducted in PubMed, Scopus, Science Direct, ISI, Web of Knowledge, and Embase using keywords relevant to the study objectives based on the PRISMA 2020 checklist.[13] All articles were reviewed until April 2023. Keywords and the MeSH terms:

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((((((("Breast Neoplasms"[Mesh] OR "Triple Negative Breast Neoplasms"[Mesh]) OR ( "Triple Negative Breast Neoplasms/surgery"[Mesh] OR "Triple Negative Breast Neoplasms/therapy"[Mesh] )) AND "Nanoparticles"[Mesh]) AND "RNA"[Mesh]) AND "MicroRNAs"[Mesh]) OR "Biomarkers"[Mesh]) OR "Biomarkers, Tumor"[Mesh]) AND "Stem Cells"[Mesh]) AND ( "Therapeutics"[Mesh] OR "therapy" [Subheading] OR "Treatment Outcome"[Mesh] ).

***Data collection***

First, a checklist was prepared, including the author's name, publication year, study design, sample, Cell Culture, therapeutic type, stem cells, miR-RNA type, nanoparticles, and therapeutic effects. The study data were entered in this checklist and summarized in Table 1. The mean differences in results were extracted and used for meta-analysis. Two independent, blinded reviewers screened each record, and a third person retrieved each report. The selection of articles was based on inclusion and exclusion criteria.

**Table 1. Full-text information extracted from selected studies.**

| Study. Years | Study Design | Animal Model | Sample Size | Control Group | Therapeutic Group | Sample | Stem Cells | MiR-RNA Type | Nanoparticles | Evaluation of Therapeutic Effects |
|---|---|---|---|---|---|---|---|---|---|---|
| Guo et al., 2022 [15] | In-vitro and in vivo | Mice | 6 | PBS | 3WJ/CD133apt/anti-miR21 nanoparticles | Human TNBC cell | CD133 | MiR21 | 400 nm | Histological analysis of active caspase 3 activity in tumor tissues |
| Yin et al., 2019 [11] | In-vitro and in vivo | Mice | 6 | PBS | 3WJ/CD133apt/anti-miR21 nanoparticles | Human TNBC cell line MDA-MB-231 | CD133 | MiR21 | 400 nm | Histological analysis of active caspase 3 activity in tumor tissues |

***Inclusion and exclusion criteria***

Only articles published in English, miRNA model based on qRT-PCR data, included. Case studies, case reports, and review articles; studies without access to the full text were excluded from the study.

***Risk assessment***

Each study was reviewed with 14 items, and the parameters were reported as yes or no using modified CONSORT Criteria (Guidelines for reporting pre-clinical in vitro studies on dental materials). These items were:

A summary of the trial design, methods, results, and conclusions, scientific background and explanation of the rationale, specific objectives and hypotheses, the intervention of each group, including how the intervention was administered, with sufficient information for replication, and completely defined. Predefined primary and secondary outcome measures. This includes how and when they were assessed, how the sample size was calculated, how the random allocation sequence was generated, the mechanism by which the random allocation sequence was implemented, who generated the random allocation sequence, who was blinded to the intervention, statistical methods used to compare groups, results for each group, estimated effects and precisions, trial limitations, addressing potential bias, imprecision, and, in the case of multiple analysis, funding sources, and other support, where access to the full trial protocol may be found.

***Data analysis***

The $I^2$ coefficient was used for assessing potential heterogeneity in studies. Values of 50%< indicate low heterogeneity, 50% to 75% indicate moderate heterogeneity, and values >75% indicate high heterogeneity. A fixed effect model with an inverse-variance method was used to calculate the effect size (95% confidence interval). Meta-analysis was conducted using STATA/MP. V17 software.

## 3. Results

***Study selection***

In the initial search, 103 articles were found based on keywords, and all articles were entered into EndNote X8 software; Duplicate articles, articles with inappropriate and inconsistent titles, and other reasons were removed, then the abstracts of 78 articles were reviewed, 67 articles were removed (based on the inclusion and exclusion criteria), and the full text of 11 articles was reviewed. Articles whose full text was incomplete had incomplete data, articles that were not in line with the objectives of the study were excluded, and finally, two articles were selected (Fig. 1). All the steps of searching and reviewing the articles were done by two blind observers and evaluated by a third observer.

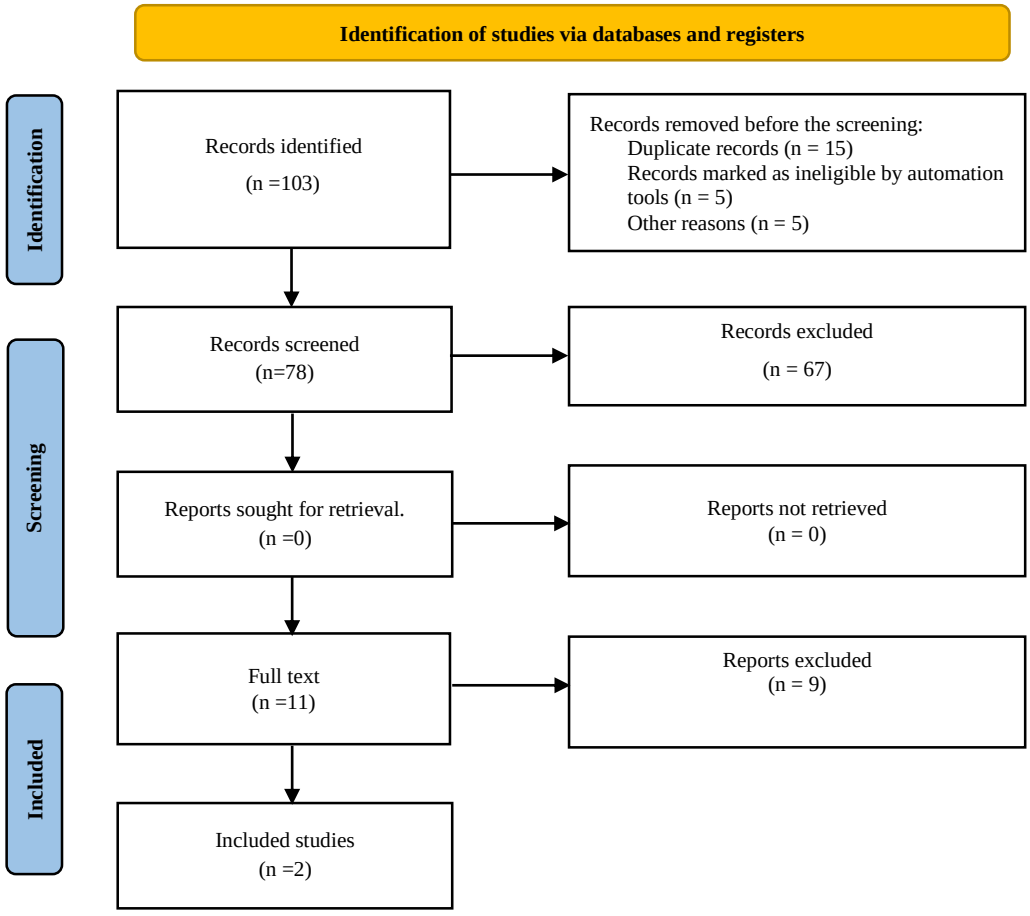


**Fig. 1. PRISMA 2020 Checklist.**

The average hydrodynamic diameter of 3WJ/CD133apt/anti-miR21 nanoparticles was 10.30 nm (ES, 10.30 nm 95% CI 7.10 nm, 13.51nm) with low heterogeneity ($I^2$=0%; P =0.86) (Fig. 2). This average is in the optimal range of the size scale, which has facilitated the effect of enhanced permeability and retention and can prevent rapid renal clearance.

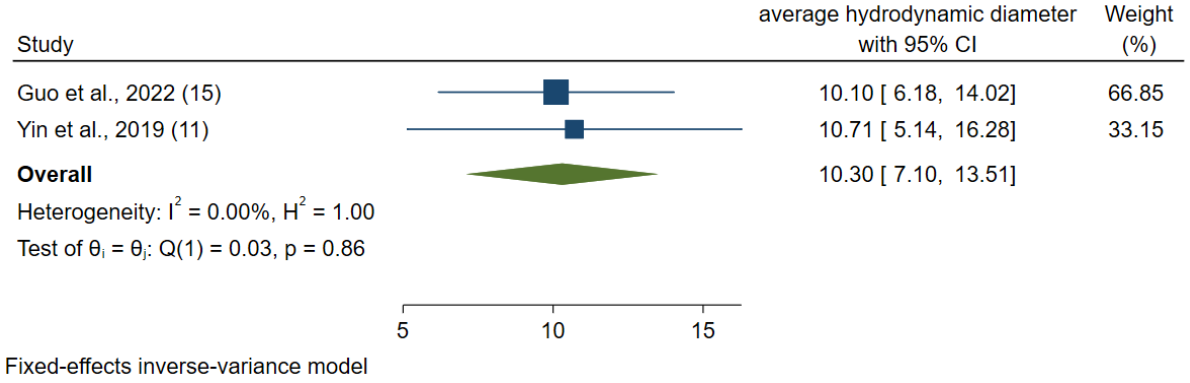


**Fig. 2. The forest plot showed the Size distribution of 3WJ/CD133apt/anti-miR21 nanoparticles.**

Zeta potential was determined to be -25.19 mV (ES, -25.19 mV 95% CI -39.57 mV, -10.81 mV) with low heterogeneity ($I^2$=0%; P =0.99) (Fig. 3). Due to the negative charge of the cell membrane; the negative sign indicates that the anionic property of RNA nanoparticles leads to the reduction of non-specific entry into normal cells.

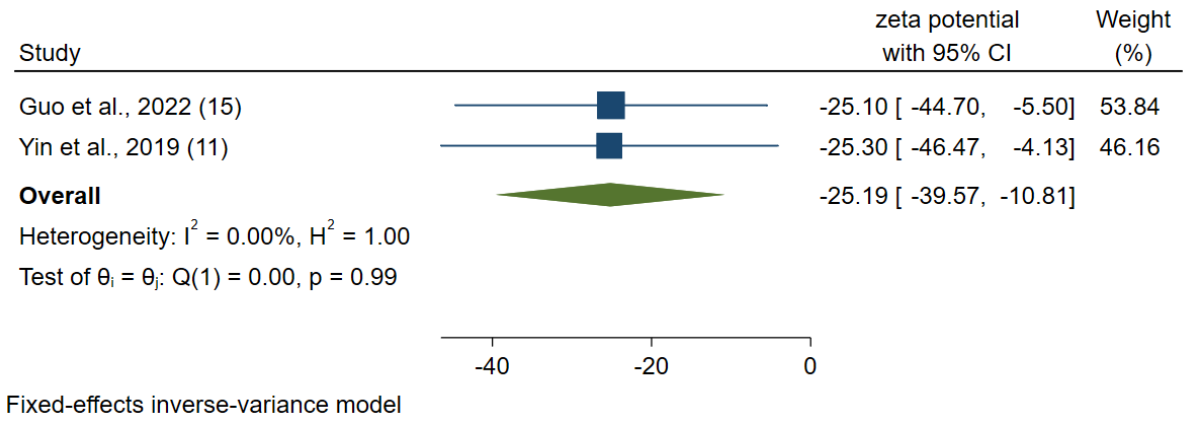


**Fig. 3. The forest plot showed the Zeta potential of 3WJ/CD133apt/anti-miR21 nanoparticles.**

In both studies,[11, 15] the temperature of 3WJ/CD133apt/antimiR21 nanoparticles was higher than 60°C, which shows that this treatment has high stability. In the comparison of two groups of mice under the study,[11] it was observed that 3WJ/CD133apt/anti-miR21 nanoparticles had a higher binding efficiency on MDA-MB-231 cells compared to the control group (Fig. 4).

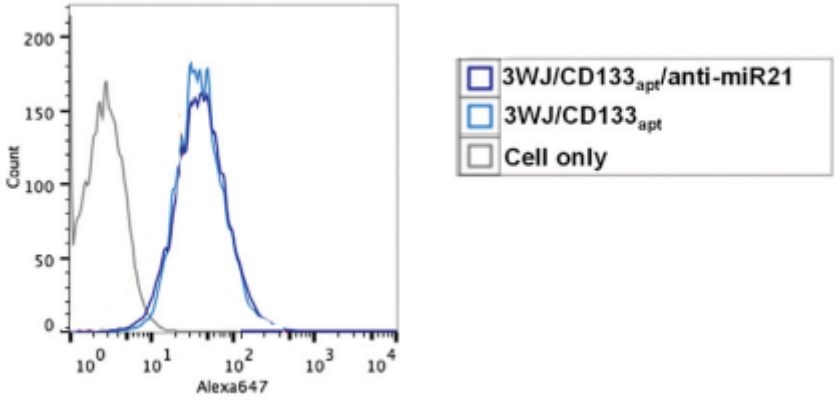


**Fig. 4. 3WJ/CD133apt/anti-miR21 at a therapeutic concentration (1 mM)(11)**

Meta-analysis showed that delivery of anti-miR21 by CD133 aptamer induced a higher Renilla to Firefly luciferase ratio in MDA-MB-231 TNBC cells in a dose-dependent manner, indicating that CD133 aptamer is a favorable mediator; (MD, 1.61 95% CI 0.54, 2.68) with low heterogeneity ($I^2$=0%; P =0.92); a significant difference was observed between the two groups (Fig. 5).

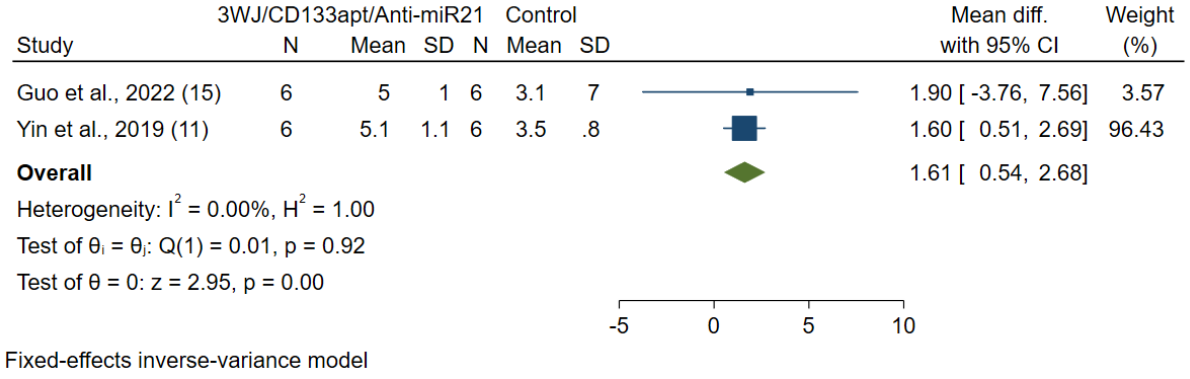


**Fig. 5. The forest plot showed the therapeutic effects of 3WJ/CD133apt/Anti-miR21 nanoparticles.**

In both studies,[11, 15] noticeable positive changes in PTEN and PDCD4 were observed after treatment with 3WJ/CD133apt/Anti-miR21 nanoparticles. Based on a sub-group meta-analysis, it was observed that 3WJ/CD133apt/Anti-miR21 nanoparticles in therapeutic concentration (1 mM) compared to the control group (lipopolysaccharide) did not induce TNF-a or IL-6 production ($p<0.05$) (Fig. 6). Based on the findings of Yin et al. 2019[11] study, nanoparticle treatment significantly inhibited tumor growth in the MDA-MB-231 xenograft model compared to the control group (Fig. 7).

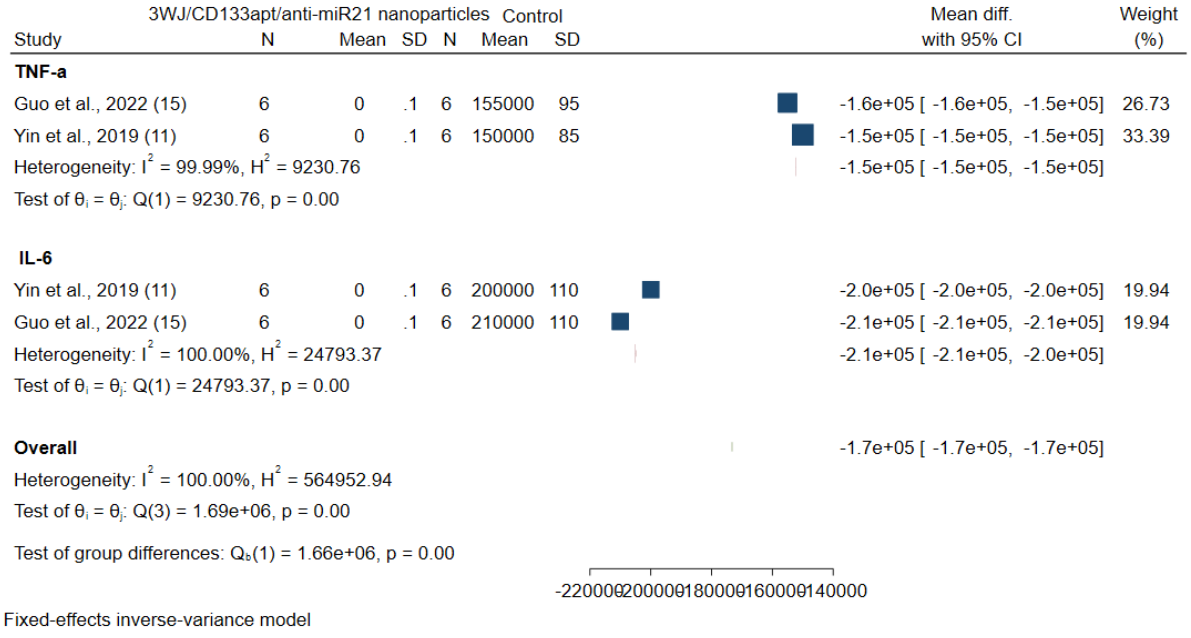


**Fig. 6. The forest plot showed TNF-a and IL-6 induction in RAW264.7 cells after treatment.**

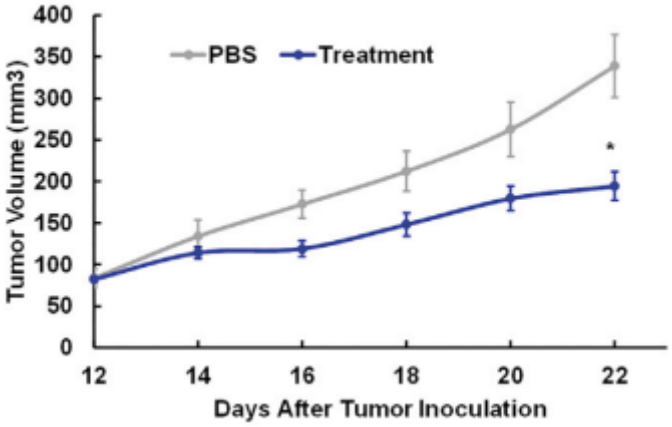


**Fig. 7. Triple-negative breast cancer tumor growth.**

## 4. Discussion

TNBC treatment has a low survival rate. As a result, it is hoped that by using new treatments, a practical step can be taken to increase the survival of patients. In the selected studies, the stability of the 20 F-modified pRNA-3WJ motif significantly improved performance. The present meta-analysis showed that the defined zeta potential and size for RNA nanoparticles (400 nm) are appropriate. The selected studies showed that the effective reduction of miR21 can lead to more expression of PTEN and PDCD4 and thus induce cell apoptosis.[11, 15] It was observed that RNA nanoparticles have a negative charge to minimize cell absorption; This feature doubles the importance of using RNA nanoparticles. Also, the small size of nanoparticles makes them enter the cells through endocytosis. Another advantage of using nanoparticles is that they can penetrate tumors by affecting EPR. The present meta-analysis showed that 3WJ/CD133apt/Anti-miR21 nanoparticles did not induce inflammation-related cytokines, TNF-a, and IL-6. Several factors are effective on the immunogenicity of RNA nanoparticles, including RNA nanoparticles are composed of ribonucleotides, cationic nanoparticles have a greater tendency to stimulate the immune system than anionic nanoparticles, and small sizes of RNA nanoparticles.[16]

## 5. Conclusion

The present meta-analysis showed that 3WJ/CD133apt/anti-miR21 nanoparticles could be considered as a suitable option in the selection of treatment for TNBC; More studies are needed to confirm the current evidence because only two studies were found that investigated the effect of 3WJ/CD133apt/anti-miR21 nanoparticles in the treatment of TNBC. It was also observed that 3WJ/CD133apt/anti-miR21 nanoparticles effectively inhibit TNBC tumor growth and target TNBC tumors in an animal model.

## Conflict of Interest

The authors declared that there is no conflict of interest.

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