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Review Article
https://doi.org/10.63501/xyvdbe23

Efficacy of Nanoparticle-Based Localized Immunomodulation to Reduce Graft-versus-host Disease in Murine Models of Bone Marrow Transplantation: A Systematic Review

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ABSTRACT:

Background and Aim: Bone marrow transplantation (BMT), which accounts for 73.8% of all transplants, is a cornerstone therapy for various hematological disorders. It follows a life-threatening complication, acute graft vs host disease (aGVHD) with an incidence of 30-60%. Despite being standard, conventional immunosuppressive therapies are frequently associated with toxicity, infections, and 70% of non-responding patients. A novel, highly targeted nanoparticle-based strategy, while being explored in solid organ transplantation, remains under-investigated in BMT. This systematic review aims to evaluate the efficacy of NP-based strategies to mitigate aGVHD following BMT.

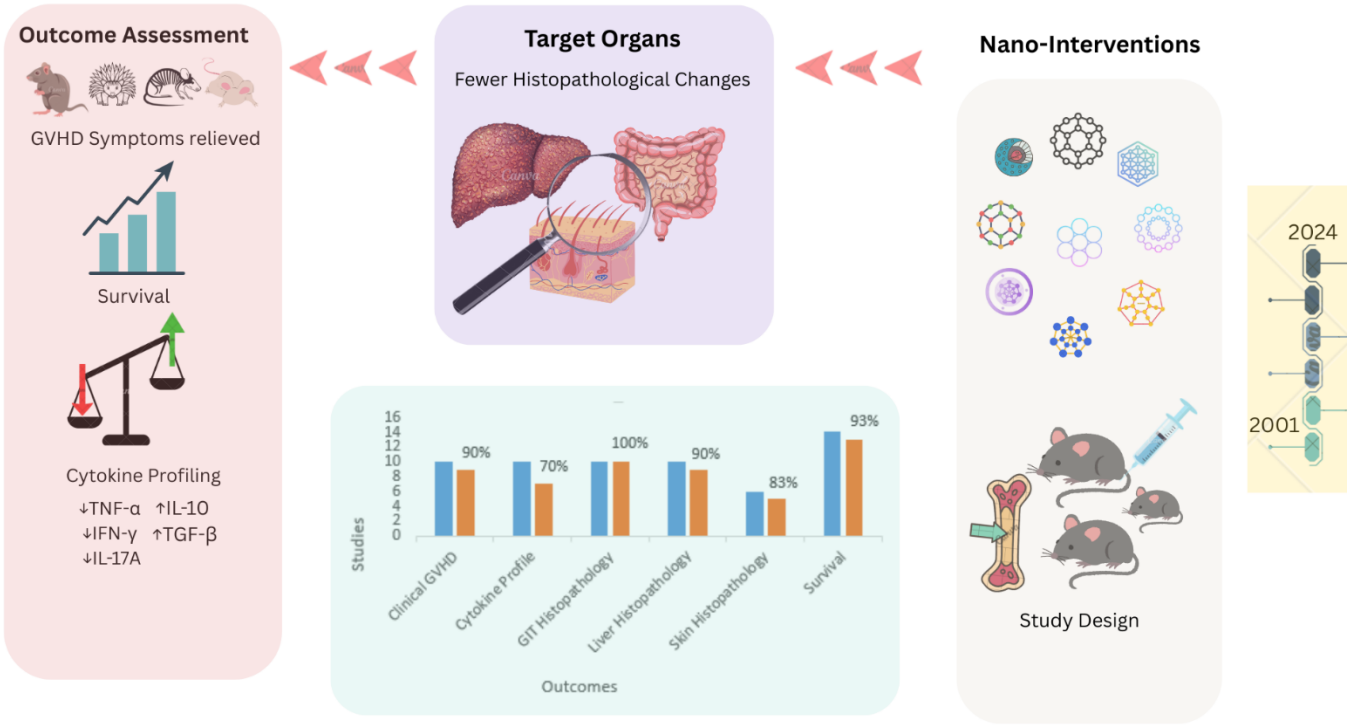
Methods: A systematic search was conducted using PubMed, Cochrane Library, and Science Direct from April 23, 2001, to August 13, 2024, for propensity-matched studies evaluating the efficacy of NP-based therapies to mitigate GVHD severity in murine models. Two reviewers independently extracted data. Study quality was assessed using the SYRCLE tool. Descriptive analysis was performed as meta-analysis was not possible due to heterogeneity in intervention types.

Results: Of 66 studies, 15 were included with n/group = 2-30; interventions being given through i.v/i.p route. Overall, the risk of bias was moderate. NP treatment lowered clinical GVHD scores by 90%($p<0.001$ for ≈ 40%), prolonged survival rates by 93%($p<0.001$ for ≈ 21%), lower histopathological tissue damage scores in the liver (90%, $p<0.001$ for ≈ 20%),), intestine (100%, $p<0.001$ for ≈ 20%), skin (83%, $p<0.001$ for ≈ 17%) and inflammatory cytokines was lowered by 70%($p<0.01$ for ≈ 30%).

Conclusion: NPs therapy has a promising efficacy in mitigating GVHD, with limitations of study design and heterogeneous interventions. Focus on high-quality comparative and safety evaluation pre-clinical studies is needed for optimization towards clinical trials

KEYWORDS: *Bone Marrow Transplantation, Hematopoietic Stem Cell Transplantation, Graft vs Host Disease, Nanoparticles*

Figure 1: Graphical Abstract



Line Diagram: Trends of Included Studies with Time

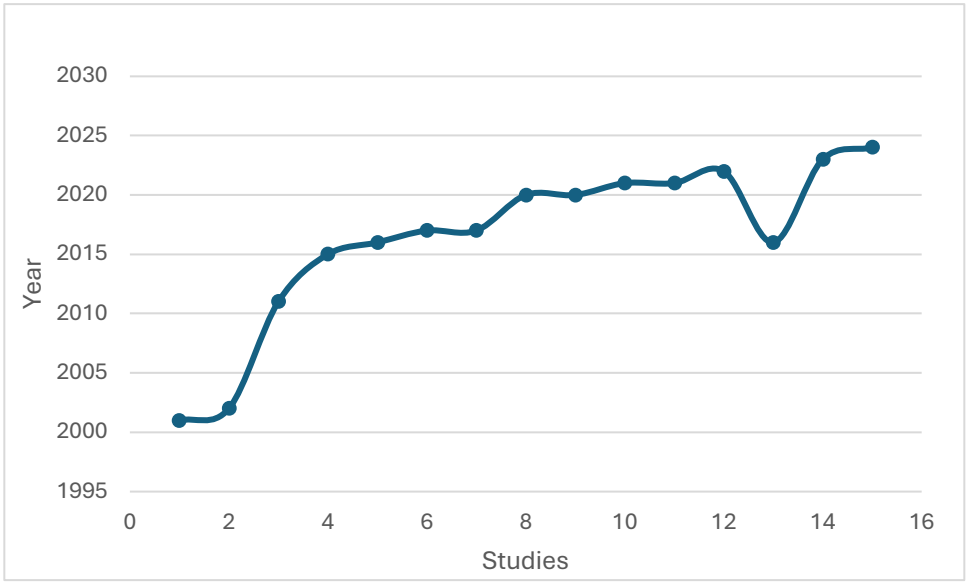
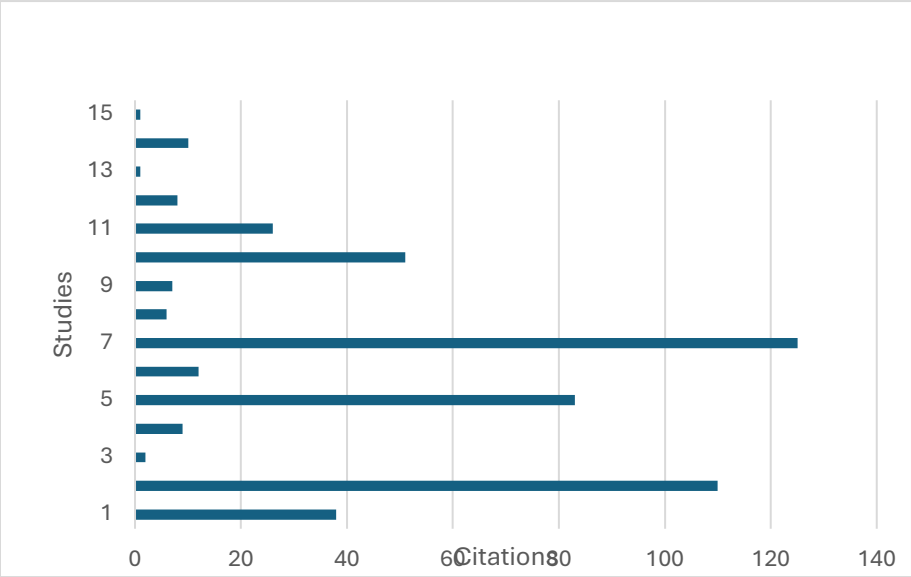


Chart 1: Citations of Included Studies



1. INTRODUCTION:

Bone marrow transplantation (BMT) is a cornerstone therapeutic intervention for a range of life-threatening conditions, including both malignant and non-malignant disorders (1). According to Western statistics, 73.8% of all transplants were allogeneic HSCT (2). Its growing application in modern medicine highlights its vital role in improving long-term survival in patients with otherwise fatal diseases (3). BMT is linked to many serious complications despite its curative potential, the most significant of which is acute Graft Versus Host Disease (aGVHD), which can complicate its medical outcome, having an immense effect on morbidity and mortality (1,4,5). The incidence of aGVHD after BMT, disease is approximately 30%-60% (6–9). aGVHD occurs when donor-derived immune cells recognize the recipient's tissues as foreign, triggering inflammatory responses and subsequent tissue damage (10). This factor significantly compromises the overall success rate of BMT and limits its effectiveness (10).

Currently, standard conventional treatments for aGVHD include systemic immunosuppressive therapy that attenuates these immune responses. Despite being common, it is associated with systemic toxicity and increased risk of serious infections. This occurs due to the suppression of the body's standard immune system (11,12). Of note, up to 70% of patients don't respond to systemic immunosuppressive therapies (6,13–16).

Now, the utmost need of the hour is to overcome the side effects associated with these conventional treatments. For this, several novel therapeutic strategies have emerged that offer localized immunomodulation. Among these nanoparticle-based drug delivery systems, one presents a promising approach for targeted tissue delivery, thereby minimizing systemic immunosuppression (17). They are linked to enhance bioavailability, prolonged drug circulation time, and specific site drug delivery, which minimizes systemic exposure and toxicity (3,17,18). Moreover, given the high cost and complications

associated with long-term immunosuppressive therapy in resource-constrained healthcare systems, nanoparticle-mediated local immunotherapy could offer a cost-effective and safer alternative (18).

Nanoparticles can also be engineered to modulate the immune responses at various levels, including antigen presentation, T-cell activation, and cytokine release (19–21). This immunomodulatory capacity of nanoparticles can be utilized efficiently in post-transplant care to enhance transplant success by preventing aGVHD and reducing the risk of disease relapse (22). Recent preclinical and early-phase clinical studies in this field have yielded encouraging data with nanoparticle-based formulations targeting the immune system components that can be potentially used in transplant immunity (23,24). Despite recent developments in this field, the integration of nanoparticles in BMT immunomodulation is still in its early stages and requires further investigation. Existing literature needs to be systematically reviewed and critically evaluated to identify and understand the current landscape of nanoparticle-based immunotherapies in BMT. This systematic review aims to critically assess and synthesize current evidence on the efficacy of nanoparticle-based strategies for localized immunomodulation in bone marrow transplantation, with a specific focus on their effectiveness, mechanisms, safety, and translational potential. By consolidating findings from preclinical studies, this review aims to identify knowledge gaps and provide guidance for future research to accelerate the integration of nanotechnology in post-transplant care.

2. METHODOLOGY:

We prospectively preregistered the protocol on the Open Science Framework (OSF DOI: https://doi.org/10.17605/OSF.IO/2HA96; registration: https://archive.org/details/osf-registrations-2ha96-v1). A comprehensive search of Cochrane CENTRAL, PubMed/MEDLINE, Web of Science, ScienceDirect, CINAHL, and Google Scholar (2000–2025) was conducted using predefined MeSH/keyword combinations for bone marrow/hematopoietic stem-cell transplantation, graft-versus-host disease (GVHD), and nanoparticles; a reviewer refined the Boolean string. Records were deduplicated in Rayyan, and two reviewers independently screened titles/abstracts and full texts against prespecified criteria, with a third resolving disagreements.

Eligible studies were peer-reviewed, English-language preclinical murine allogeneic BMT models with experimentally induced acute GVHD that evaluated intravenously or intraperitoneally administered nanoparticle interventions (e.g., drug delivery, immune-cell/biopolymer carriers) versus conventional immunosuppression, empty nanoparticles, or no treatment; the primary outcome was GVHD mitigation. We excluded non-murine or autologous models, studies without induced aGVHD or primarily focused on GVL, gray/unpublished literature, and non-empirical articles, as well as non-IV/IP administration routes and combination-therapy designs that precluded an explicit comparator.

Of 66 records, 15 duplicates were removed; 51 underwent screening, 36 were excluded, and 15 studies were included. Two reviewers independently extracted research, animal, intervention, comparator, and outcome details into a standardized spreadsheet; a third reviewer adjudicated discrepancies. Risk of bias was assessed independently by two reviewers using the SYRCLE tool, with consensus resolution for conflicts.

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Table 1: Study Characteristics of Included Studies

Identification			Methods	Population						Interventions					Comparison	Outcomes	
Author	Year	Country	Study design	Species	Strain	Sex	Age	Sample Size	Inclusion criteria: GVHD Induced Models	Housing Conditions	Intervention	Route	Dose	Frequency	Timings	Comparator Groups	Outcome Measures	Follow-Up Duration
Bernardes et al., 2015	2015	Brazil	Pre-clinical animal study	Mouse	C57BL/6 × DBA/2 (B6D2F1)	*	8–12 weeks	5	Low-dose irradiation + splenocytes	Temperature-controlled (23 ± 1 °C), 12h light/dark	Fullerol NPs	i.p	10 mg/kg	14	Every 48 hours for 28days post-transplant	PBS-treated GVHD	GVHD (clinical scoring, survival, weight loss, histopathologies)	47 days
M0069a & Saxena, 2020	2020	India	Pre-clinical animal study	Mouse	C57bl/6 and F1 (C57bl/6 x Balb/c F1)	*	8–12 weeks	3	Splenocytes from parental strain	SPF, 25 ± 1 °C, 50–60% RH	AF-SWCNTs	i.v	50 µg per mouse	aGVHD-1 & aGVHD-2 = 4	aGVHD-1 & aGVHD-2 = Day 2,4,6,8	PBS-treated (vehicle)	GVHD (clinical scoring, survival, weight loss, histopathologies)	8–10 days
Zhao et al., 2023	2023	United States	Pre-clinical animal study	Mouse	BALB/c and C57BL/6	*	8–12 weeks	10	Irradiation + splenocytes and bone marrow	Pathogen-free	Chitosan-Alginate Nanoencapsulated T cells	i.v	1.0 × 10^6	1	Day 0	Non-encapsulated T cells (control) + BMC control	GVHD (clinical scoring, survival, weight loss, histopathologies)	70 Days
Cheng et al., 2011	2011	China	Pre-clinical animal study	Mouse	BABL/c and male C57BL/6	M+F	10-12 weeks	10	Irradiation + bone marrow/splenocytes	Not specified	Fe_3O_4 MNPs + CsA	i.p	600 mg/kg/day	25	One Daily for 25 days post-transplant	CsA only group, Fe_3O_4 MNPs only group Irradiated control, Irradiation-only group	GVHD (clinical scoring, survival, weight loss, histopathologies)	28 days
Pareek et al., 2022	2022	United States	Pre-clinical animal study	Mouse	BALB/c and C57BL/6	F	6-8 weeks	10	Irradiation of 8Gy + Bone Marrow cells with/without Splenocytes	*	BRNPs	i.v	10 mg/kg	5	One daily 0-4 days post-transplant	GVHD model (BM + splenocytes) without BRNPs &BM only group	GVHD (clinical scoring, survival, weight loss, histopathologies)	90 days
Jiang et al., 2023	2023	China	Pre-clinical animal study	Mouse	C57BL/6 and BALB/c	M+F	8–10 weeks	*	Irradiation of 8Gy + BM cells + Splenocytes	pathogen-free conditions	Nanosized MSC-derived exosomes	i.v	300µg/ml	3	3 times in a week post-transplant	Total body irradiation(control group), BMCs+SCs+PBS(PBS group), BMCs+SCs+MSC(MSC control group)	GVHD (clinical scoring, survival, weight loss, histopathologies of liver, skin GIT)	40 Days
Tina K. Kaiser et al., 2020	2020	Germany	Pre-clinical animal study	Mouse	C57BL/6 and BALB/c WT	*	8–12 weeks.	3-23 per group	Irradiation of 8.5 Gy + BM cells + T cells	specific-pathogen-free conditions	GCs on BMP-NPs vehicle	i.p	10 mg/kg	Short-term: 3; Long-term, 6	Short-term: day 3,4,5; Long-term, Day 3,4,5,7,9,12	BM only, PBS control, EP-NP control	GVHD (clinical scores, survival, histopathology og GIT, inflammatory cytokine profile)	Short-term: 6 days; Long-term 40-50 days
Weijiang Liu et al., 2021	2021	China	Pre-clinical animal study	Mouse	BALB/c & C57BL/6j	M+F	6-8 Weeks	≥ 3	800 cGy TBI + BM Cells+ Splenocytes	specific-pathogen-free conditions	MSCs-exosomes contain functional miR-223	i.v	MSCs 1 × 106	1	Day 0	PBS Control+ aGVHD group	GVHD (clinical scores, survival, histopathologies of liver & GIT, inflammatory cytokine profile)	20 Days

Review Article
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Przybylski S et. Al., 2017	2017	Germany	Pre-clinical animal study	Mouse	C57Bl/6wt & Balb/cwt	M	8–12 Weeks	2-16 per group	Irradiation of 8G + BM cells + splenocytes	specific-pathogen-free conditions	PEI	i.v	50 µg	1	Day 0	GVHD control + Ibafect treated control	GVHD (clinical scores, survival, inflammatory cytokine profile)	60 Days
Kuroiwa T et. Al., 2001	2001	Japan	Pre-clinical animal study	Mouse	C57BL/6 and (B6 × DBA/2)F1	*	8–12 weeks	5 per group	900 cGy TBI + BM Cells + Splenocytes	specific-pathogen-free conditions	HGF-HVJ on liposomes vehicle	i.m	8 µg	4	Day 0, 7, 14, 21	GVHD control	GVHD (clinical scoring, survival, weight loss, histopathologies)	90 Days
Mei D et al., 2024	2024	China	Pre-clinical animal study	Mouse	C57BL/6 & BALB/c (H2d)	F	8–10 weeks	*	busulfan (25 mg/kg/days) and cyclophosphamide (125 mg/kg/days) + BM Cells + Splenocytes	Specific Pathogen Free	encapsulated donor T-cell	i.v	2×10^6 cells per mouse	1	Day 0	NC group, BMCs group, BMCs+T cell transplant group	GVHD (clinical scores, survival, histopathologies of liver, skin, GIT, inflammatory cytokine profile)	70 days
Wang L et. Al., 2016	2016	China	Pre-clinical animal study	Mouse	BALB/c and C57BL/6	M	8-10 week	10-15 per group	7.5 Gy TBI + BM Cells + Splenocytes	specific pathogen-free	hUC-MSC-Evs	i.v	200 µg of hUC-MSC-EVs,	2	Day 0 & 7 post-transplant	PBS Control	GVHD (Clinical scores, survival, Histopathologies of liver, skin, GIT & inflammatory cytokines profiles)	60 Days
Fujii S, et al., 2017	2017	Japan	Pre-clinical animal study	Mouse	C57BL/6 C57BL/6	*	7 to 9 weeks	5-30 per group	8 Gy TBI + splenocytes	Specific pathogen-free	EVs derived from human BM-MSCs	i.v	2 x 10^6 human BM-MSCs	1	Day 5 post-transplant	PBS Control	GVHD(clinical scores, survival, histopathologies of liver, skin, GIT, inflammatory cytokine profile)	100 Days
Yi Zhang et. Al., 2002	2002	New Haven	Pre-clinical animal study	Mouse	C3H.SW & C57BL/6	*	*	5 to 8	Irradiation of 9.5 Gy + Spleen cells with/without BM Cells	Sterile conditions	Lipo-chlodronate	i.v	150µl	1	Day -1 pre-Transplant	Liposomes only (control)	GVHD (clinical scores, survival, histopathology of liver and skin, inflammatory cytokine profile)	66 Days
Ke-Liang L et al., 2021	2021	China	Pre-clinical animal study	Mouse	e C57BL/6 and BALB/c	M	6–8 weeks	10	Irradiation of 8GY + BM Cells+ Splenocytes	*	Msc-exo	i.v	200 µg exosome	1	Day 0	IL-10 & anti-TNF-α drug etanercept Treated Controls	GVHD (clinical scores, survival, inflammatory cytokine profile)	30 Days

Abbreviations: aGVHD / cGVHD, Acute / Chronic Graft Versus Host Disease; BM, Bone Marrow; BRNPs, Bilirubin Nanoparticles; CsA, Cyclosporine A; F1, First Filial Generation; GVHD, Graft Versus Host Disease; GVL, Graft Versus Leukemia; MNPs, Magnetic Nanoparticles; NPs, Nanoparticles; RH, Relative Humidity; SPF, Specific Pathogen Free; HSCT, Hematopoietic stem cell transplantation; BMC, Bone marrow control; CTL, Cytotoxic T Lymphocyte ; P, Primary; S, Secondary; i.v, Intra-venous; i.p, Intra-peritonea; i.m, Intramuscular; F, Female; M, Male; PBS, Phosphate Buffer Saline; Gy, Gray; BMP-NPs, Hybrid Nanoparticles, GC, Glucocorticoids; MSC, mesenchymal stem cells; BMZ, free betamethasone; EP, Empty; BMP; TBI, Total body irradiation; PEI, polyethyleneimine; exo, exosomes; HGF, Hepatic growth factor; HVJ, Hemagglutinating virus of Japan; EVs, Extracellular vesicles; hUC-MSC-EVs, extracellular vesicles released from human umbilical cord-derived MSCs; GIT, Gastrointestinal tract; * Not Reported; _, Not applicable

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3. RESULTS:

A total of 66 records were identified through multi-database electronic research. After removing 15 duplicate articles, 51 records remained for screening. Based on the title and abstract, 31 records were excluded. The remaining 18 full-text articles were assessed for eligibility, and finally, 15 studies, reported across 16 publications, were included in this review. The study selection process and reasons for exclusion are detailed in the PRISMA flow diagram given below (Figure 4).

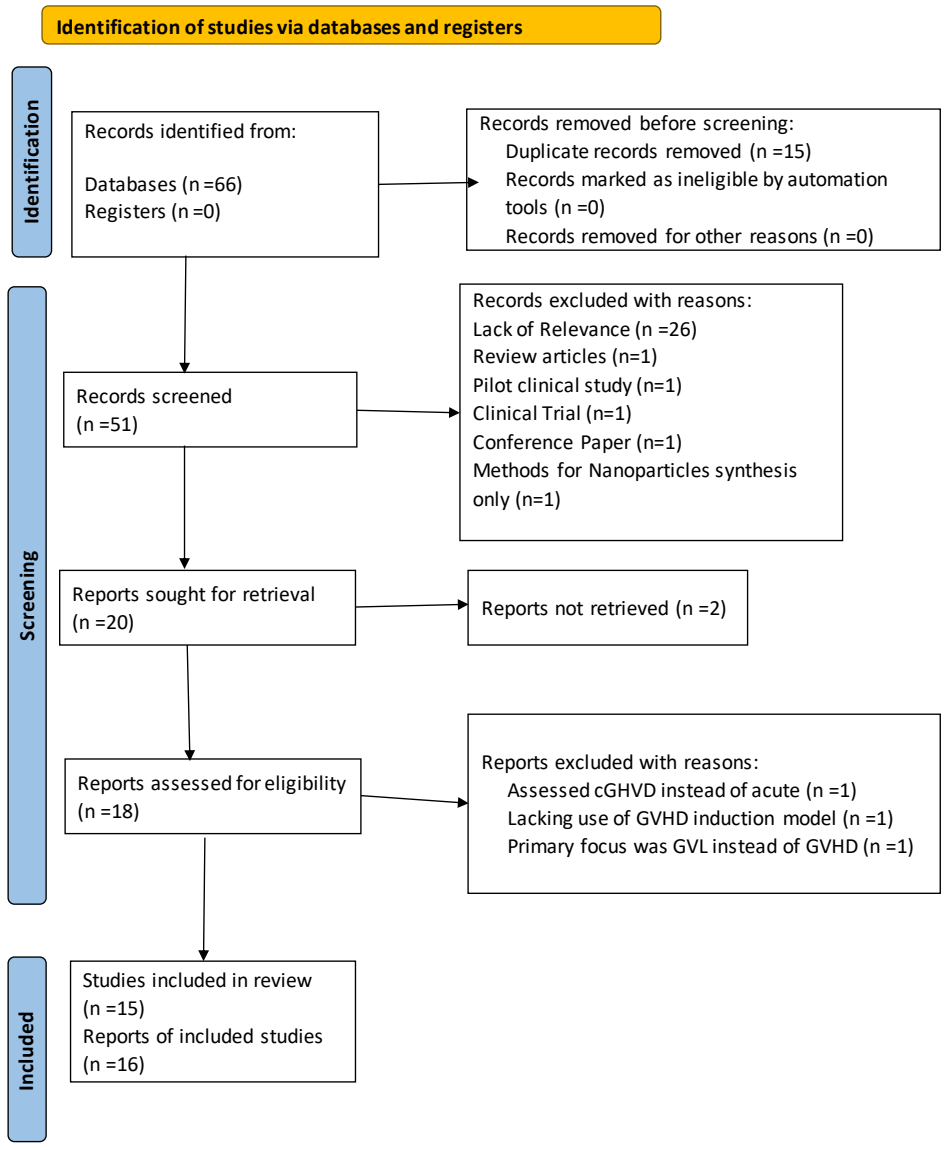

Fig. 4. PRISMA 2020 Flow Diagram for the Systematic Review
This PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram illustrates the process of identifying, screening, and including studies for the systematic review and meta-analyses. The diagram consist of four main phases: Identification, Screening, Eligibility, and Inclusion. The number of studies involved in each phase are shown in figure.

https://doi.org/10.63501/xyvdbe23

3.1 GVHD Assessment

3.1.1 GVHD Clinical Scoring

Clinical symptoms, such as weight loss, changes in posture and activity, fur texture, diarrhea, skin integrity, and fecal occult blood, are typically graded to assess the severity of acute GVHD. 10 out of 15 included studies reported this outcome. A scale of standard scoring system for determining the clinical manifestations of aGVHD was employed in each study. The scale assigned grades of 0 to 2 for each clinical parameter regarding severity: grade 0 for normal, grade 1 for mild to moderate, and grade 2 for severe. Authors then summed up the grades for each criterion to calculate the overall effect size of the intervention. Low clinical scores were expected to have a large effect size.

Table 2: Material-by Mechanism

Intervention Type	**Mechanism**	**Target Organs**
Fullerol NPs	Scavenges ROS, ↓cytokines	Liver, intestine
BRNPs	↓ ROS and TNF-α/IFN-γ	Systemic
AF-SWCNTs	Uptake by T/B cells; depletion	Systemic
Lipo-chlodronate	Depletes hepatic/splenic macrophages & DCs; ↓ $CD8^+$ recruitment	Spleen, lymphoid tissues
Chitosan–alginate encapsulation	Blocks donor T-cell contact with host APCs	Liver, gut
Gelatin–alginate capsules	Inhibits co-stimulation (CD28–CD80, etc.)	Skin, lung, liver
HGF-HVJ liposomes	↓ APCs; ↓ antigen presentation	Small intestine
MNP-CsA	Targets CsA to lymphoid tissues	Spleen, liver, intestine
BMP-NPs + GCs	↓ local intestinal cytokine signaling	Liver, intestine
PEI NPs	Gene modulation; ↓ inflammatory pathways	Liver, spleen
MSC-exo	Anti-inflammatory miRNAs/proteins ↓ Th1/Th17	Systemic; leukemia (AML) sites
MSC-exo (miR-223)	↓ ICAM-1; reduced T-cell adhesion/migration	Intestine, liver, skin
hUC-MSC-EVs	↓Th1/Th17,↓ Tregs	Secondary lymphoid tissues
BM-MSC EVs	Activate iNKT, expand Tregs, ↓ alloreactive T cells	Liver, spleen
Fullerol NPs	Scavenges ROS, ↓ cytokines	Spleen; systemic cytokines

Abbreviations: BRNPs, Bilirubin Nanoparticles; CsA, Cyclosporine A; MNPs, Magnetic Nanoparticles; Evs, Extracellular vesicles; MSCs, Mesenchymal stem cells; BM, Bone marrow; hUC, Human umbilical cord; NPs, Nanoparticles; HGF, Hepatocyte growth factor; HVJ, Hemagglutinating virus of Japan

9/10 studies (23,28–35) – 90% – reporting this outcome showed a combined general trend of low clinical scores, with a statistically significant difference between the intervention group and the controls. For example, in a study (36), authors employed a technique of nano-encapsulation of T cells for localized immunomodulation. 1x10^6 Chitosan-Alginate Nanoencapsulated T cells were given through the i.v route

at day 0, the day of transplant, and followed up for 70 days. 6 Clinical Parameters, such as weight loss, posture, activity, fur texture, diarrhea, and skin integrity, were assessed. The maximum score was 12 according to the scale above. Compared to mice transplanted with control non-encapsulated T cells, with a mean score of 9.7 ± 0.4 out of 12, the mice transplanted with encapsulated T cells developed less severe GVHD symptoms, including ruffled fur, fur loss, hunching, weight loss, reduced activity, and diarrhea, with a mean score of 4.3 ± 0.5 out of 12. The difference between mean scores was ~5.4 points with $p < 0.01$. Treatment with the Nanoencapsulation strategy essentially reduces the chance of developing cutaneous GVHD.

Another study (37) utilized human BM-MSC-derived EVs with a nanosize. The clinical scores of treated mice were lower than those of the control mice, with an average of 2.8 versus 3.5. Consistent with the above, this finding showed a moderate link between nanoparticle-based therapies and the amelioration of systemic symptoms of acute GVHD.

A study (MNPs) of the intervention brought no apparent changes in clinical symptoms mitigation between the treated and control groups; statistically non-significant results (p>0.05). Findings from GVHD Clinical Scoring are summarized in Table 3.

Table3: Summary of Clinical GVHD Scoring

Intervention Type	**p-value**	**Effect Size with %age**
Rx 2, 3, 14, 15	<.001	*** 40%
Rx 5, 7 ,11	<.01	** 30%
Rx 1, 4	<.05	* 20%
Rx 8	>.05	ns 10%

n/group=**5-28**

3.1.2 Weight Loss

Weight loss is a big concern for patients undergoing BMT. This weight loss can have dangerous consequences, potentially enhancing the risk of mortality. 14/15 (93%) reported studies showed a statistically significant reduction in weight loss (24, 28–34, 36–41).

A study (33) reported that weight loss greater than 10% was considered indicative of significant GVHD. Hepatocyte growth factor liposomal treatment had no significant effect on the body weights of the recipients for 4 weeks after the induction of GVHD. Still, it prevented subsequent weight loss in the surviving mice during a 90-day observation period.

3.1.3 Survival Outcomes

GVHD has an impact on patient survival and long-term outcomes. Knowing these survival outcomes and evaluating for prognostic factors is a prerequisite for GVHD management. Fourteen studies (24,28–34,36–41) reported this outcome measure. Thirteen studies – 93% – showed a positive association between the intervention and survival, with a mean follow-up duration of 52 days (range, 6-100).

For example, in a study (40), authors observed mice for 66 days and reported that 21.1% (n=19) of lipo-clodronate-treated mice died from acute GVHD, compared to 60.7% (n=23) of controls, with $p < 0.01$. Lipo-chlodronate treatment essentially reduces mortality rates. Another study (37) found that GVHD mice receiving systemic infusions of extracellular vesicles derived from Mesenchymal stem cells exhibited prolonged survival, with a median survival of 16 days compared to 10 days in control GVHD mice over a 100-day follow-up period. This intervention probably enhances survival.

Table 4: Summary of Survival Outcomes

Intervention Type	p-value	Effect size with %age
Rx 4, 10, 14	<.001	*** 21%
Rx 2, 3, 5, 6, 11, 12	<.01	** 50%
Rx 1, 7, 9	<.05	* 21%
Rx 8	>.05	ns 7%

n/group=**4-32**

In study (24), recipient mice with bone marrow (BM) transplant only survived throughout the experiment; 100% survival of BM cells was observed in controls. Mice infused with a mixture of splenocytes and bone marrow cells resulted in significant GVHD-related mortality; 40% survival in BM cell + Splenocyte (HR 5.898, p=0.0134). Mice in the treatment group showed 90% survival (BM cell + Splenocyte + BRNP Treatment) with (HR 0.2055, p=0.0112). It suggested that attacking the early inflammation cascade with bilirubin nanoparticles has therapeutic benefits in BMT. Bilirubin Nanoparticles possess a strong positive correlation with survival outcomes. Findings from Survival Rates are summarized in Table 4.

3.1.4 Tissue Histopathology

GVHD assessment typically involves histopathology to assess organ damage. Histopathology plays an essential role in diagnosing and staging GVHD. Liver, gastrointestinal tract, and skin are the most commonly affected organs in aGVHD.

3.1.4.1 Liver Histopathology

Ten studies (23,29–32,34,37,39–41) showed a statistically significant decline in liver tissue damage, including hepatocyte edema and necrosis, massive inflammatory cell infiltration, fibrosis, and disorganized liver lobules in nine studies (90%).

For example, in a study (36), GVHD pathological scoring was performed according to a standard scoring system. Six parameters were evaluated in the liver: portal inflammation, percentage of portal tracts involved, bile duct damage, lobular inflammation, bile duct loss, and fibrosis in each section. 0 indicates normal, and 1, 2, and 3 represent mild, moderate, and severe tissue damage caused by donor T cells, respectively. The grading scheme consisted of ordinal categories ranging from "0" (no effect) to "4" (severe effect) as follows. (1) Portal inflammation and lobular inflammation: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. (2) Fibrosis: 0 = none, 1 = portal, 2 = periportal, 3 = bridging fibrosis, and 4 = cirrhosis. Portal inflammation, bile duct damage, lobular inflammation, and fibrosis were detected in mice treated with non-encapsulated T cells, and 100% of portal tract inflammation was observed. Portal Inflammation scores for non-encapsulated were 3.3 (100%), compared to 1.5 for encapsulated; lobular Inflammation scores were 3.2 for non-encapsulated and 1.5 for encapsulated; and fibrosis scores were 0.5 for non-encapsulated and 0 for encapsulated. The scores in recipient mice transplanted with encapsulated T cells were significantly lower than those in mice transplanted with non-encapsulated T cells ($p < 0.05$), suggesting a large effect size of this intervention in minimizing liver damage.

In another study (33), authors observed that the total number of hepatic mononuclear cells was markedly increased from 0.5 × 10^6 to 2.0 × 10^6 after 2 weeks of GVHD. HGF treatment decreased the total number of hepatic mononuclear cells from 2.0 × 10^6 to 1.4 × 10^6 cells at 2 weeks, and it significantly reduced the number of donor T cells from 1.1 × 10^6 to 0.3 × 10^6 cells, representing a 55% reduction per liver. These results indicate that HGF treatment essentially inhibits donor T-cell infiltration into the liver and subsequent hepatic injury. Findings from Liver Histopathology are summarized in Table 5.

Table 5: Liver Histopathology Summary

Intervention Type	**p-values**	**Effect Size with %age**
Rx 3, 4	<.001	*** 20%
Rx 5, 6	<.01	** 10%
Rx 1, 7, 8, 9, 10	<.05	* 60%
Rx 11	>.05	ns 10%

n/group =2-32

3.1.4.2 Intestinal Histopathology

Ten studies (24,30–34,37–39,41) reported this outcome, and all (100%) showed a statistically significant decline in intestinal tissue damage. For example, study (41) stated that bacterial translocation aggravates GVHD and can result in sepsis and ultimately in patient death. The number of bacterial colony-forming units (CFUs) in the peritoneal cavities of mice with experimental GVHD and treated with fullerol was considerably reduced compared with that of untreated mice. The reduced bacterial translocation in mice with experimental GVHD, treated with fullerol, correlated with the preservation of intestinal parenchyma. A score was generated according to the aforementioned criteria in the liver section. By summing the changes, the maximum score index was 9. The authors observed a score of 6 with $p < 0.05$ in the treated group. Fullerol Nanoparticles treatment may prevent intestinal damage.

In study (24), single cell necrosis per view in 40x object was observed and had $p = 0.0029$. The authors suggested that bilirubin nanoparticle treatment peri-transplantation could prevent histopathologic GVHD of the intestine. Findings from GIT Histopathology are summarized in Table 6.

Table 6: Summary GIT Histopathology

Intervention Type	**p-values**	**Effect Size with %age**
Rx 3,4	<.001	*** 20%
Rx 1, 2, 5, 13	<.01	** 40%
Rx 8, 9, 10, 11	<.05	* 40%

n/group =2-32

3.1.4.3 Skin Histopathology

Six studies assessed skin histopathologies (23,30,31,33,41). Five studies (83%) showed a positive correlation between the treatment groups and the prevention of pathological damage in the skin. For instance, in a study (37) about the skin, the percentage of mice with severe damage was lower in the extracellular vesicles-treated group than in the control group (60.0% vs. 88.8%). This nano-based therapy probably prevents the pathological damage in the skin. Findings from Skin Histopathology are summarized in Table 7.

3.1.5 Inflammatory Cytokine Levels

GVHD assessment in bone marrow transplantation involves tracking immune modulation and cytokine effects. These play vital roles in its development and advancement. GVHD occurs when donor immune cells (T cells) attack the recipient's tissues. Measuring cytokine levels, such as TNF-α, IL-6, IFN-γ, IL-2, and IL-10, helps assess the activity of GVHD.

Table 7: Summary of Skin Histopathology		
Intervention Type	**p - values**	**Effect Size with %age**
Rx 3	<.001	*** **16.7%**
Rx 5	<.01	** **16.7%**
Rx 1, 8, 11	<.05	* **50%**
Rx 6	>.05	ns **16.7%**

n/group =10-23

Ten studies (23,28–32,38–41) reported this outcome with a consistent trend of low cytokine profile. 7/10 studies showed positive findings. For example, in a study (39), the inflammatory improvement in aGvHD mice caused by miR-223 was assessed by measuring the expression of proinflammatory factors in serum. The expression of IFN-γ, IL-17A, and TNF-α was lower in the miR-223Agomir-treated group (1468.4 ± 59.8, 49.78 ± 16.8, and 49.9 ± 4.5) than that in the aGvHD group (2773.3 ± 28.4, 342.8 ± 192.2, and 329.2 ± 60.3) or negative control group (2463.1 ± 59.9, 174.3 ± 62.3, and 232.3 ± 15.6) (Fig. 5d). These findings indicated that miR-223 essentially reduced the expression of proinflammatory factors in aGvHD mice. Findings from Inflammatory Cytokine Levels are summarized in Table 8. Cumulative Studies with Positive Findings are given in Chart 2. The Cross-Domain Heat Map Effect is shown in Table 9. The overall efficacy ranking is calculated in Chart 3.

Table 8: Summary of Inflammatory Cytokines Levels		
Intervention Type	**p-Values**	**Effect Size with %age**
Rx 3, 4, 5	<.01	** **30%**
Rx 1, 9, 10, 14	<.05	* **40%**
Rx 2, 7, 13	>.05	ns **30%**

n/group =2-15

Abbreviations: BRNPs, Bilirubin Nanoparticles; CsA, Cyclosporine A; MNPs, Magnetic Nanoparticles; EVs, Extracellular vesicles; MSCs, Mesencymal stem cells; BM, Bone marrow; hUC, Human umblical cord; NPs, Nanoparticles; HGF, Hepatocyte growth factor; HVJ, Hemagglutinating virus of Japan; AF-SWCNTs; Acid functionalized single wall carbon nanotubes; n, Sample size; ***, Highly significant; **, Moderately significant; *, Significant; ns, Not significant.

Treatment Code: Rx 1= BRNPs, Rx 2 = GCs, Rx 3 = hUC-MSC-EVs, Rx 4 = MSC-exosomes (miR-223), Rx 5 = Nanosized MSC-derived exosomes, Rx 6 = Lipo-clodronate, Rx 7 = Chitosan-Alginate Nanoencapsulated T cells, Rx 8 = Fe_3O_4 MNPs + CsA, Rx 9 = Fullerol NPs, Rx 10 = HGF-HVJ liposomes, Rx 11 = EVs (BM-MSCs), Rx 12 = PEI, Rx 13 = Encapsulated donor T cells, Rx 14 = Msc-exo, Rx 15 = AF-SWCNTs
Note: Limited and highly significant findings have been highlighted in red and Green respectively.

https://doi.org/10.63501/xyvdbe23

Table 9: Cross-Domian Heat Map Effect

Treatment Code	Clinical GVHD	Survival Rates	Liver Histo.	GIT Histo.	Skin Histo.	Inflam. Cytokines
Rx 1	*	*	*	**	*	*
Rx 2	***	**		**		ns
Rx 3	***	**	***	***	***	**
Rx 4	*	***	***	***		**
Rx 5	**	**	**	**	**	**
Rx 6		**	**		ns	
Rx 7	**	*	*			ns
Rx 8	ns	ns	*	*	*	
Rx 9		*	*	*		*
Rx 10		***	*	*		*
Rx 11	**	**	ns	*	*	
Rx 12		**				
Rx 13				**		ns
Rx 14	***	***				*
Rx 15	***					

Effect Sizes: ***, Highly significant; **, Moderately significant; *, Significant; ns, Not significant

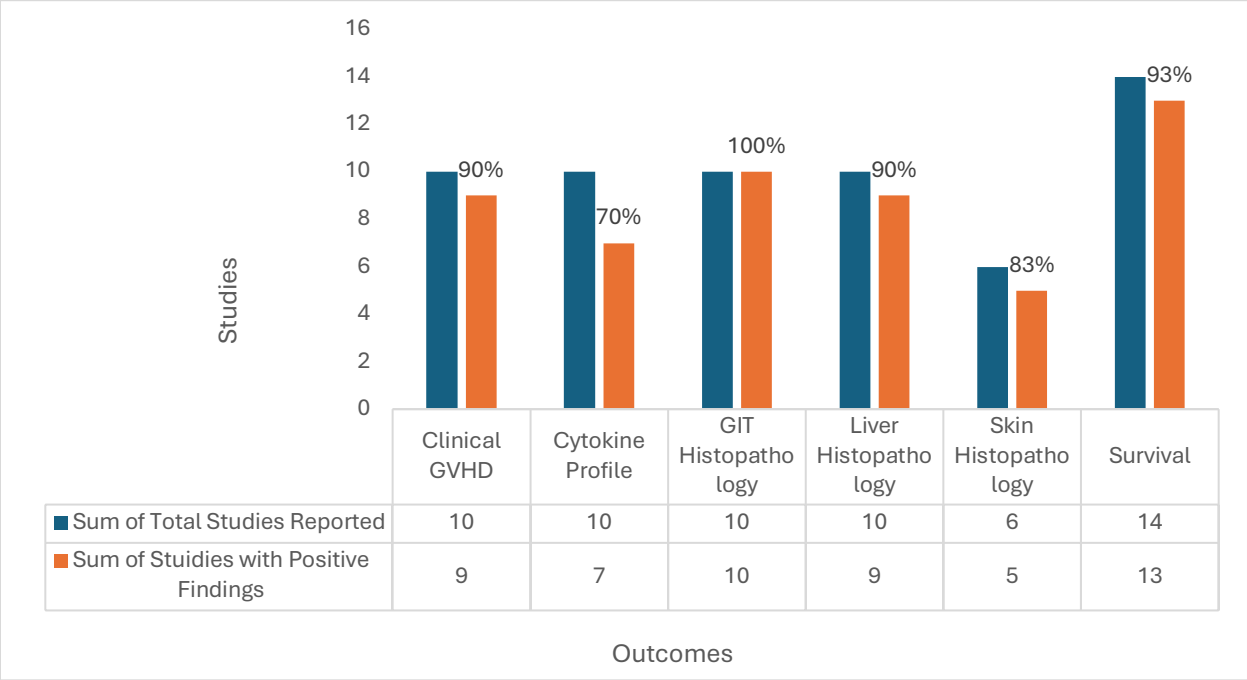

Chart 2: Cumulative Studies with Positive Findings

3.2 Risk of Bias Assessment

Applying the SYRCLE tool's (27) judgment criteria, we assessed that all studies were adequately reported in the baseline characteristics domain and avoided selective reporting (15/15; 100%). In contrast, the following domains, sequence generation, allocation concealment, random housing, caregiver blinding, and random outcome assessment, were not disclosed satisfactorily in any study (15/15; 100%). Details about blinding of outcome assessors were sufficient in 8 out of 15 (53%) studies. No Incomplete outcome data were identified in all but one study (14/15; 93%).

Overall, the reporting quality of the studies was moderate, with several domains lacking a precise methodological detail. Only one study was judged to be at high risk of bias in the critical domain of random outcome assessment (1/15; 7%). Findings from this study were considered with caution when interpreting overall efficacy trends. These gaps suggest potential performance and detection bias, which may reduce confidence in outcomes such as histopathology scoring. A domain-wise summary of risk of bias is presented in Table 10.

Table 10: Risk of Bias Assessment

Study ID	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Overall
Bernardes et al., 2015	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Zhao et al., 2016	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Pareek et al., 2022	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Mia & Saxena,2020	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Cheng et al., 2011	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Jiang et al., 2023	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Tina K. et al., 2020	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Weijiang Liu et al., 2021	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Low	Moderate
Przybylski S et. Al., 2017	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Moderate
Yi Zhang et. Al., 2002	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Kuroiwa T et. Al., 2001	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Lai et al., 2018	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Wang L et. Al., 2016	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Fujii S, et al., 2017	Moderate	Low	Moderate	Moderate	Moderate	Moderate	Moderate	Low	Low	Low	Moderate
Ke-Liang L et al., 2021	Moderate	Low	Moderate	Moderate	Moderate	High	Low	Low	Low	Low	High

D1 Sequence Generation
D2 Baseline characteristics
D3 Allocation concealment
D4 Random housing
D5 Blinding of caregivers
D6 Random outcome assessment
D7 Blinding of assessors
D8 Incomplete outcome data
D9 Selective reporting

Low Risk
Moderate Risk
High Risk

4. DISCUSSION

From Table 9 and Chart 3, it is evident that the efficacy of different NP interventions in mitigating aGVHD varies considerably across domains. hUC-MSC-EVs treatment achieved the highest efficacy index, aligning with a similar study (42), which highlights the findings of reduced pro-inflammatory cytokine expression and demonstrates that encapsulation techniques significantly enhanced the treatment's protective effects. HGF-HVJ (miR-223) consistently demonstrated a strong multi-domain protection, which is corroborated by a similar study (43). Fullerol NPs and nanosized MSC-derived exosomes also demonstrated broad and substantial benefits, particularly in reducing inflammatory cytokines and tissue pathology, as shown in a study (44).

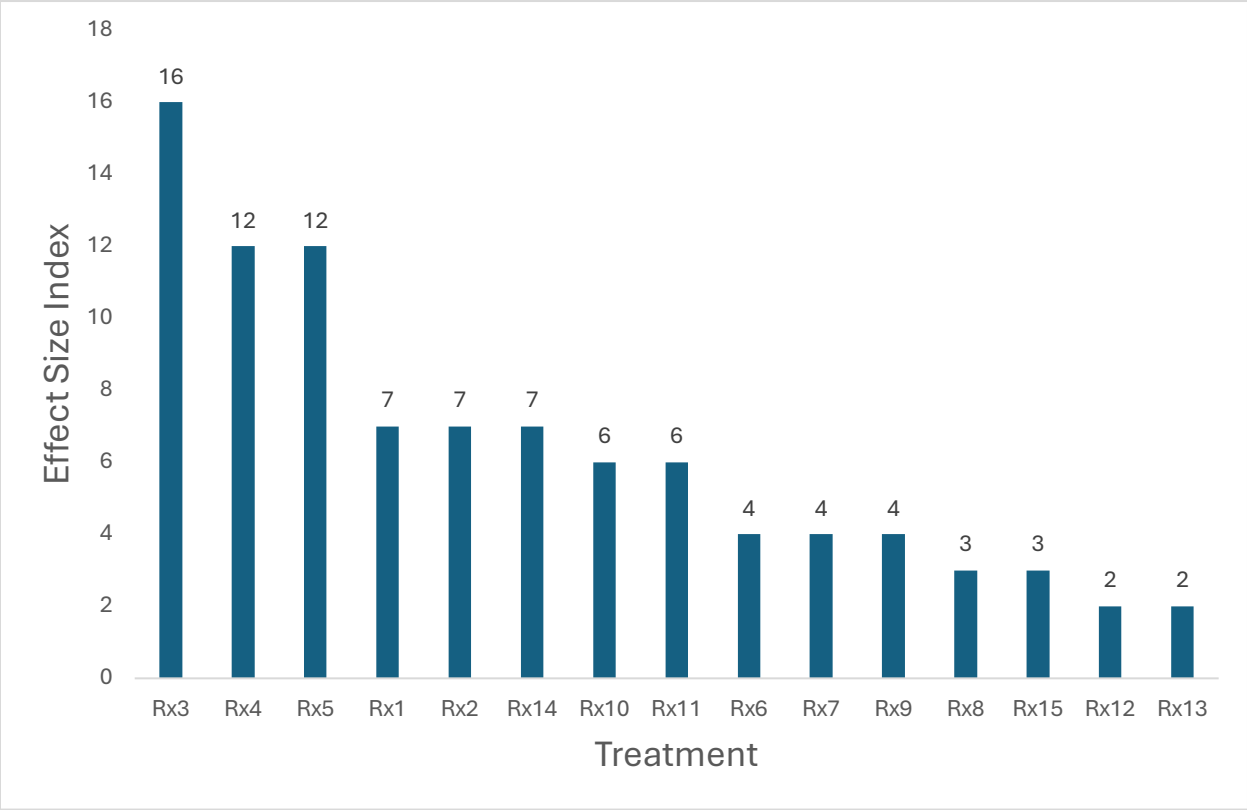

Chart 3: Overall Efficacy Ranking

Table 9, Chart 3: Treatment Code: Rx 1= BRNPs, Rx 2 = GCs, Rx 3 = hUC-MSC-EVs, Rx 4 = MSC-exosomes (miR-223), Rx 5 = Nanosized MSC-derived exosomes, Rx 6 = Lipo-clodronate, Rx 7 = Chitosan-Alginate Nanoencapsulated T cells, Rx 8 = Fe_3O_4 MNPs + CsA, Rx 9 = Fullerol NPs, Rx 10 = HGF-HVJ liposomes, Rx 11 = EVs (BM-MSCs), Rx 12 = PEI, Rx 13 = Encapsulated donor T cells, Rx 14 = Msc-exo, Rx 15 = AF-SWCNTs
Note: Effect size index has been calculated by considering 1*=1

In contrast, the findings of PEI and Fe_3O_4 MNPs + CsA were constrained, indicating limited efficacy, which aligns with the study (45). Collectively, these findings highlight that NP-mediated delivery provides a potent therapeutic efficacy across all domains. Our findings confirm the validity, robustness, and highly targeted immune regulation of the novel treatment. Compared to previous narrative reviews, this study provides a unique mechanistic map by linking each material type to the underlying immunological mechanism.

Although nanoparticle applications are expanding rapidly, they still face challenges in their translational aspects. Since 2019, only a few NPs have been approved by the FDA or the European Medicines Agency (EMA) (46). We should focus on nanoparticle platforms that facilitate the use of FDA-approved drugs (47). Additionally, no active targeting NPs have progressed beyond clinical trials (48). Due to their more complex design, they face more challenges in scale-up production (48). To make it more reliable, they require more characterization steps and a longer development timeline, which increases costs (48). The following are emerging technologies that can aid in characterizing single nanoparticles (49). *Nanopore-based technologies:* Label-free, single-molecule nanopore sensing has been expanding for the sequencing of nucleic acids and protein-based analytes (50–53).

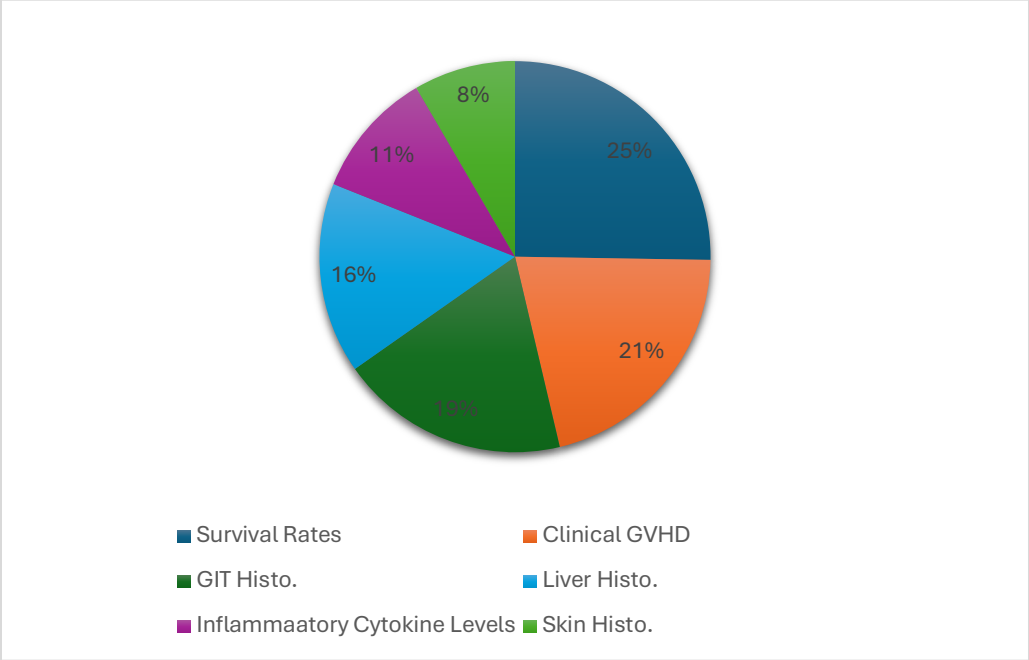

Chart4: Cumulative Efficacy in Individual Domain

In cytotoxicity profiling, dose-dependent cytotoxicity and genotoxicity have been observed in vitro and in vivo (54,55). Future studies should also focus on in-depth toxicology studies. One such solution is better characterization of NPs (54,55). Current quality control for nanoparticles has no standardized physicochemical characterization (56). Another study demonstrated that traditional techniques used to record the amounts of drug content are inconsistent and can lead to discrepancies in reported data (57). Control of nanomaterials can help achieve repeatability, determine progress efficiency, and ensure product safety (58). Recently, in the clinical domain, the most effective COVID-19 vaccines, to date, utilize nanotechnology to deliver immunostimulatory mRNA. However, their high cost equates to low affordability (59). Exploration should be conducted for biocompatible and biodegradable materials that are locally abundant, easily accessible, and can be used to produce nanoparticles in local labs for mass production. One such biomaterial, Chitosan nanoparticles, has been studied in one of our included studies. These can be extracted even from house flies' chitin. However, our findings suggest that its efficacy is limited. Their intrinsic biocompatibility positions them as promising candidates for innovative solutions in medical interventions (60).

The number of patents about nanotechnology has increased significantly between 2000 and 2024 (61,62). Fortunately, we have an economic framework for such in-depth studies, as countries such as the US, China, and members of the European Union (EU) have integrated nanotechnology into their national strategies (63, 64). Thus, future studies must vigorously focus on methods to enhance their scientific validity, efficacy, and safety levels.

5. CONCLUSION

With calibration, these systematic review findings suggest that NPs have a reasonably good efficacy in reducing aGVHD. However, current findings are limited by reliance on mouse models, moderate risk of bias, and heterogeneity of interventions. Future work should focus on high-quality pre-clinical studies, direct comparisons with conventional immunosuppressants, and long-term safety evaluations, followed by early-phase clinical trials. The future of nanotechnology will be influenced by ongoing collaboration among academic institutions, industry leaders, and governments.

6. Funding

The authors received no specific funding for this work

7. Conflicts of Interest

The authors declare that there are no conflicts of interest.

8. Ethical Approval

Ethical approval was not required for this study, as the review was based entirely on previously published data and did not involve any new experiments.

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List of Abbreviations

- **aGVHD / cGVHD** – Acute / Chronic Graft Versus Host Disease
- **allo-HSCT** – Allogeneic Hematopoietic Stem Cell Transplantation
- **AF-SWCNTs** – Acid-Functionalized Single-Walled Carbon Nanotubes
- **BM** – Bone Marrow
- **BMC** – Bone Marrow Control
- **BMZ** – Free Betamethasone
- **BMP** – *Not Reported
- **BMP-NPs** – Hybrid Nanoparticles
- **BM** – Bone Marrow
- **BMT** – Bone Marrow Transplantation
- **BRNPs** – Bilirubin Nanoparticles
- **CsA** – Cyclosporine A / Cyclosporin A
- **CTL** – Cytotoxic T Lymphocyte
- **EP** – Empty
- **F** – Female
- **F1** – First Filial Generation
- **GCs** – Glucocorticoids
- **GVHD** – Graft Versus Host Disease
- **GVL** – Graft Versus Leukemia
- **Gy** – Gray
- **HSCT** – Hematopoietic Stem Cell Transplantation
- **i.p** – Intraperitoneal
- **i.v** – Intravenous
- **IFN-γ** – Interferon-gamma
- **M** – Male
- **MNPs** – Magnetic Nanoparticles
- **MSC** – Mesenchymal Stem Cells
- **NPs** – Nanoparticles
- **P** – Primary
- **PBS** – Phosphate Buffered Saline

https://doi.org/10.63501/xyvdbe23

- **RH** – Relative Humidity
- **S** – Secondary
- **SPF** – Specific Pathogen-Free
- **SYRCLE** – Systematic Review Center for Laboratory Animal Experimentation