of liver diseases [248–250]. Organoids are becoming a universal tool for advancing the understanding of the biology of liver diseases and helping to find new therapies [251]. Virus-infected organoids can also be co-cultured with other types of targeting cells. Most importantly, these culture systems lack immune cells, such as B and T cells, lymphocytes, and macrophages, which are usually present in mucosal tissues and which play a vital role in combating viral infections, such as HBV/HCV infections. The use of organoid technology can eliminate the interference of the immune system and allows independent study of other functions without the influence of the immune system. With the further development and maturity of the organoid virus toolkit, including complex co-cultivation and genetic engineering of organoid viruses, virus strains isolated from patients can be used for larger-scale drug screening. All these efforts will increase the chances of identifying a clinically successful treatment for liver diseases.

Clinical Application and Challenges for use of MSCs in Liver Diseases

Currently, many studies are registered for using MSC transplantation for liver diseases in clinical trials (www.clinicaltrials.gov), according to a data search using the terms "MSC" and "liver disease". Clinical trials using MSCs to treat liver disease are presented in Table 1. Fifty-one clinical trials are registered at the time of writing, and the corresponding ratio (including sources, types, and phases) is presented in Fig. 3. Among these, UC-MSCs (24 cases) and BM-MSCs (14 cases) are the most common cell source, but nine trials did not indicate the source (Fig. 3A). AD-MSCs and menstrual blood-derived MSCs had two and one study registrations, respectively. Although few trials are registered for using AD-MSCs and menstrual blood-derived MSCs for treating liver diseases, we conducted some modest research

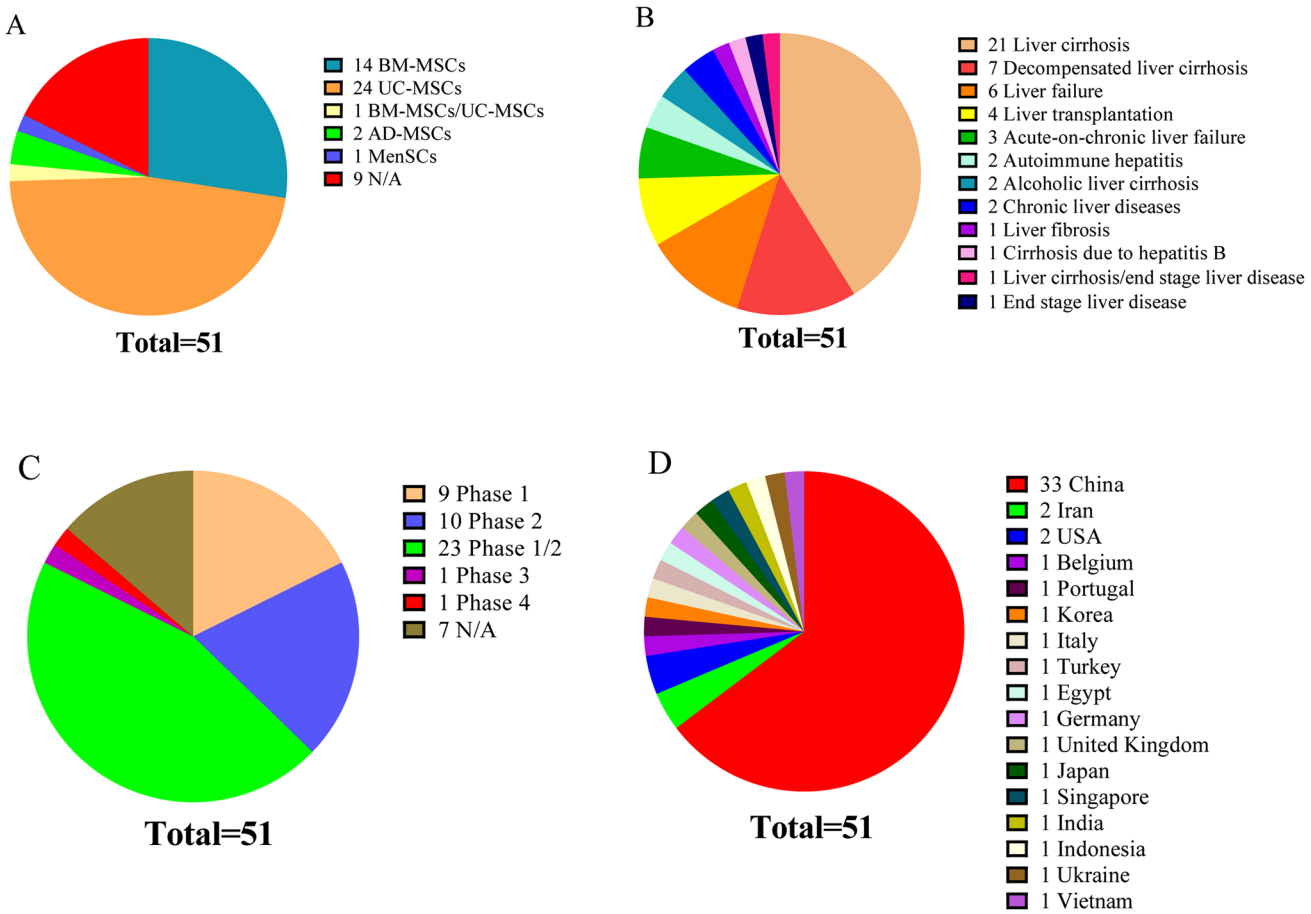

Fig. 3 Registrations for liver diseases using MSC transplantation in clinical trials. Fifty-one clinical trials were registered and the corresponding ratio including the sources (**A**), disease types (**B**), phases (**C**), and countries (**D**). Among these, UC-MSCs (24 cases) and BM-MSCs (14 cases) are the most common source. There were 21 trials for liver cirrhosis; 7 trials for decompensated liver cirrhosis; 6 trials for liver failure. Most clinical trials were conducted in phase 1 or phase 2. Most of clinical trials were registered in China (33 cases), while only two clinical trials were registered in Iran and USA

of about 30 clinical trials involving AD-MSC therapy for targeted liver damage, cirrhosis, and NASH in Japan and 60 clinical trials involving menstrual blood-derived MSC therapy for liver cirrhosis in China (data not shown). Interest in MSC-based therapy for liver diseases appears to be increasing in Japan and China. Currently, 21 trials are registered for liver cirrhosis; seven trials for DLC; six trials for liver failure; four trials for liver transplantation; three trials for ACLF; two trials for AIH, alcoholic liver cirrhosis, and chronic liver diseases; and one trial each for other diseases (Fig. 3B). Most clinical trials were phase 1 or phase 2 trials, while only two clinical trials were registered as phase 3/4 trials (Fig. 3C). Most of clinical trials were registered in China (33 cases), while only two clinical trials were registered in Iran and USA (Fig. 3D). At present, many studies have confirmed the effectiveness of MSCs in the treatment of liver diseases [103, 252, 253], but no standard protocols or perfect models for treating liver diseases are available.

MSC-based treatment requires further research and validation, including selection of optimal migration pathways, more efficient MSC sources, long-term detection of MSCs, and safety assessment of MSCs [254]. Although MSCs have important application prospects in clinical medicine partly based on anti-inflammatory and immunomodulatory properties, many issues need to be resolved [255]. To overcome the corresponding difficulties, many related improvement strategies are needed. Generally, healthy, fresh, and autologous MSCs are the best source for MSC-based therapy. Different isolation and maintenance approaches may result in different effects when these cells are used in the treatment of diseases. To date, the MSC isolation methods have mainly included the enzymatic method and explant culture method [256]. Although the explant culture method has been used to isolate MSCs only from a few tissue types, these cells should be used without adding additional proteolytic enzymes. A standard for isolation and maintenance of human MSCs is urgently needed [257]. In scientific studies, experiments are typically first conducted in mice to explore the therapeutic effect of MSCs, because of the high homology between mice and humans. It is unclear whether MSCs differentiate into HLCs in humans. Owing to the strict ethical constraints, it is not possible to obtain human liver tissue to check the markers of MSCs differentiating into HLCs. However, this is a widely recognized mechanism for animal models. Much needs to be learnt to understand the mechanisms underlying MSC-based treatment fully.

Importantly, the biosafety of MSCs needs to be studied carefully before clinical use, to rule out their effect on genetics. Due to a lack of standardized and ideal surface molecular markers, high quality and consistency of MSCs are rare. This heterogeneity is attributed to donor variability, different cell culture systems, and various environmental conditions (such as individual procedures, injection methods, epidemiological background, time, culture conditions, and the donor's age, hormonal level, and health status). In addition, the use of MSCs does not produce consistent clinical outcomes due to the limited survival of injected cells in host tissues [258]. Although most researchers have demonstrated that transplantation of MSCs (including those from different sources) is safe in clinical trials [259, 260], studies on the long-term safety or sustained treatment effects of MSCs are limited [261]. No data can guarantee their long-term safety in hosts post-MSC transplantation. In addition, the optimal dose, timing of injection, frequency of injection, and route of administration differ, making it difficult to establish a regular treatment for liver diseases. Thus, randomized controlled trials are necessary to assess the long-term safety and efficacy of MSC-based treatment with liver diseases.

Conclusions

MSC-based therapy for liver diseases from bench to bedside is illustrated in Fig. 4. Some new research hotspots warrant further exploration, such as CRISPR/CAS9-mediated gene modification of MSCs, preconditioning to enhance their function, single-cell RNA sequencing of MSCs for precision medicine, and development of organoids for three-dimensional models. Understanding these interactions will help us choose the optimal dose and appropriate method for MSC treatment of liver diseases. In summary, MSC-based strategies have great potential for ameliorating liver dysfunction, reducing mortality, and improving the quality of life of patients suffering with liver diseases. As a type of adult stem cell, the particular mechanism by which MSCs perform various functions need to be elucidated further for the purpose of regenerative medicine and clinical applications.