Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3281

IMMUNOLOGICAL SHIFTS IN HCV INFECTION: ENHANCED LIVER BIOMARKERS, CD56^{BRIGHT} NK CELLS AND IMPAIRED CYTOTOXICITY IN CD16^{DIM} SUBSET

Awais Khan[1], Mirza Ameer Faizan Ali[2], Bushra Iftikhar[3], Azka Rizvi[4], Nargis Aman[5], Shanza Rafique Malik[6], Sarwat Moon[7], Aamna Shah[8*], Khaleel Ullah[9], Mazhar Ali Khan[10*]

[1]Department of Innovative Technology of Biomedical Engineering & Medical Devices, Ming Chi University of Technology, Taiwan.
[2]Assistant Professor, Pathology at Al-Aleem Medical College, Lahore - Pakistan
[3]Assistant Professor, Biochemistry department at Azra Naheed Medical College Lahore - Pakistan
[4]Medical Technologist at Department of Microbiology, Pakistan Kidney and Liver Institute & Research Center, Lahore - Pakistan
[5]Assistant Professor, Nazar College of Pharmacy, Dukson Institute of Health Sciences, Islamabad - Pakistan
[6]Department of Microbiology, University of Haripur - Pakistan
[7]Indus Hospital and Health Network, Lahore - Pakistan
[8*]Department of Pharmacy, Women Institute of Learning and Rehabilitation Sciences, Abbottabad - Pakistan
[9]Department of Microbiology, Kohat University of Science & Technology, Kohat - Pakistan
[10]Department of Microbiology, Hazara University, Mansehra – Pakistan,
Email: mazharjalali123@gmail.com

***Corresponding author:** Aamna Shah, Mazhar Ali Khan
*Department of Pharmacy, Women institute of Learning and Rehabilitation Sciences Abbottabad – Pakistan, Email: aamnakhan2010@yahoo.com
[*]Department of Microbiology, Hazara University, Mansehra – Pakistan,
Email: mazharjalali123@gmail.com

Abstract

Background: Hepatitis C is a liver disease caused by the hepatitis C virus (HCV), which is chronic, asymptomatic, inflammatory, progressive, and slow-moving.

Objectives: The objective of the study were distribution of acute and chronic infections, treatment outcomes, immune cell levels, viral load, and gender-related disparities in liver enzyme levels.

Methodology: The study employed the Abbott PCR apparatus for real-time thermocycling, the MINDRAY BA-88A Semi-Automatic Clinical Chemistry Analyzer, and flow cytometry to analyze samples from Hepatitis C Virus (HCV) infected patients, with a specific emphasis on evaluating CD56 and CD16 markers on Natural Killer (NK) cells.

Results: This study findings highlighted 87.5% of patients exhibited liver cirrhosis, a severe condition linked to liver failure, while 12.5% had hepatocellular carcinoma. Out of 150 patients 52% had acute HCV infection, 10% had chronic infection, and 38% endured treatment. CD56+ NK cell levels averaged 21±4, with males at 22±6 and females at 18±4, suggesting gender-related differences. CD16+ NK cells averaged 9±4 overall, with males at 8±2 and females at 12±4. Viral load ranged from 62,000-65,000IU/mL (41.33%) to 310,000-418,000IU/mL (7.33%). Genotype 3a was most

prevalent (51.70%), while 1a, 2a, and 3b constituted 10.80%, 13.80%, and 17.10%, respectively. Gender disparities were noted in ALT (males: 127±8, females: 113±2), AST (males: 131±4, females: 117±1), and GGT (males: 62±3, females: 49±2) levels.

Conclusion: The study concluded that the liver cirrhosis as the predominant condition in HCV-infected patients, indicating substantial liver damage due to HCV infection. The study also emphasized the potential of HCV treatment in mitigating liver cancer risk.

Keywords: Natural Killer Cells, CD56^{Bright}, CD16^{Dim} Liver Biomarkers, Hepatitis C

Introduction

Hepatitis C is a liver disease caused by the hepatitis C virus (HCV), which is chronic, asymptomatic, inflammatory, progressive, and slow-moving (Shaz et al., 2019). It has been estimated that around 71 million people are globally affected by chronic HCV infection is a severe public health issue (Hayes et al., 2022). HCV infection leads to the dysregulation of the host's immune response and favors the development of hepatocellular carcinoma. Hepatitis C virus (HCV) is an enveloped RNA virus classified within the Flavivirus family, which also includes viruses like dengue and yellow fever (Lucchese et al., 2022).

Hepatitis C virus (HCV) can quickly explore genetic variations due to its high replication and mutation rates. The emergence of drug-resistant variants is influenced by factors such as genetic barriers to resistance, fitness of resistant populations, and extent of medication exposure. These elements collectively contribute to the development of drug-resistant HCV variants (Domovitz et al., 2022).

Natural killer (NK) cells are a vital component of the innate immune defense system. They are the primary effector cells responsible for direct cytotoxic effects, which involve antiviral activities that lead to the elimination of virus-infected cells. Additionally, NK cells play a crucial role in triggering adaptive immunity by regulating immunoregulatory cytokines. They also hinder the replication of viral complexes, contributing to the overall immune response against viral infections (Fang et al., 2022).

There is an imbalanced distribution of NK cell subsets in HCV patients, marked by an increase in the CD56bright subset and a decrease in the hypofunctional CD56-CD16+ subset that has reached a mature stage. In terms of functionality, NK cells from HCV-infected patients produce reduced amounts of tumor necrosis factor alpha (TNFa) and interferon gamma (IFN-γ), while their cytotoxic capabilities remain either normal or heightened (Wang et al., 2022).

The objective of the study is to present the key findings of a study on Hepatitis C Virus (HCV) infection, including the distribution of acute and chronic infections, treatment outcomes, immune cell levels, viral load ranges, genotype distribution, and gender-related disparities in liver enzyme levels.

Methodology

Abbott RT-PCR

The Abbott PCR appratuatus was used to constitutes a real-time thermocycling mechanism reliant on the Polymerase chain reaction (PCR) principle, facilitating the identification and quantification of ribonucleic acid (RNA) specific to the hepatitis C virus (HCV) within specimens obtained from patients. This device operates as a self-contained system, orchestrating the comprehensive workflow of real-time PCR, encompassing sample handling to the subsequent derivation of analytical results (Uzair et al.).

The process involved three key stages:

1. The patient samples were treated with a specific reagent to extract hepatitis C virus RNA (HCV RNA). The RNA was then isolated from the sample.
2. The isolated HCV RNA was converted into complementary DNA (cDNA) using a reverse transcriptase enzyme.

3. The cDNA was amplified using a PCR machine, which involved cycles of heating and cooling to denature the DNA, bind primers to target sequences, and extend the DNA strands. This results in the exponential amplification of HCV DNA.

Liver Biomarker

MINDRAY BA-88A Semi-Automatic Clinical Chemistry Analyzer was used to analyze samples from patients with Hepatitis C Virus (HCV) infection. We focused on quantifying key liver function markers Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Gamma-Glutamyl Transferase (GGT). The BA-88A Analyzer, designed for clinical chemistry analysis, facilitated the precise measurement of these markers in a semi-automated manner. This approach allowed us to gain valuable insights into the liver's health and biochemical status in HCV-positive patients (Bilbeisi et al., 2023).

Natural Killer Cells profiling by Flow Cytometry

Flow cytometry was used to analyze samples from Hepatitis C Virus (HCV) positive patients. Our main focus was on assessing Natural Killer (NK) cells, specifically their CD56 and CD16 markers. Flow cytometry is a technique that allows us to examine and quantify different cell populations within samples. We employed fluorescently labeled antibodies to stain CD56 and CD16 markers on NK cells. The flow cytometer, an advanced instrument, then measured the fluorescent signals emitted by these markers as cells passed through laser beams (Tembhare et al., 2022).

Inclusion Criteria

Only HCV positive patients including Acute and chronic.

Exclusion Criteria

We excluded the co-infected patients, liver transplant patients and pregnant women.

Graphic and Statistical analysis

SAS software was used for comprehensive statistical analysis, which involved percentages, means, standard deviations, and determining the significance of differences through p-values.

Results

1. Demographics

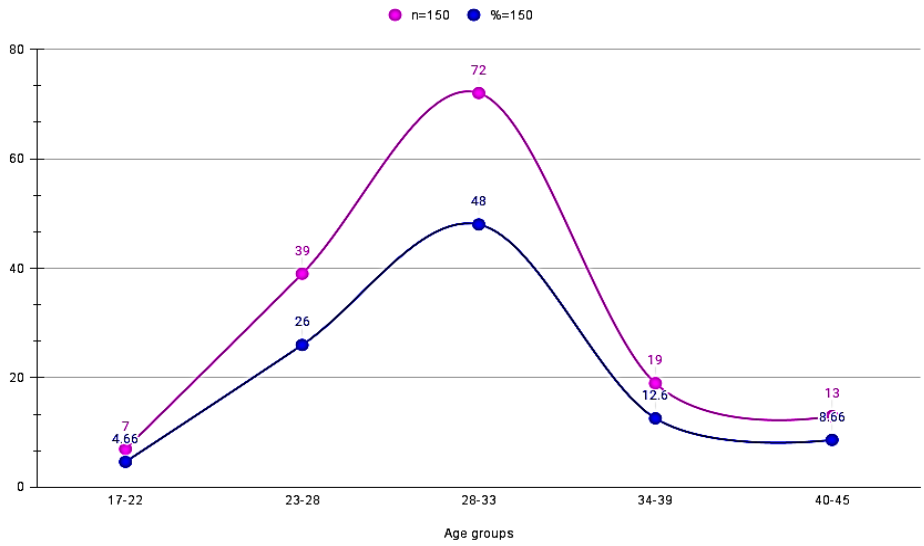

Figure 1: Participation of HCV patients based on Age-groups

Table 1: HCV Positive Patient Profile, Distribution and Condition Breakdown

Specific characteristics of patients (n=72)	N	%	P-value
Liver Cirrhosis	63	87.5	
Hepatocellular carcinoma	9	12.5	<0.05
Stage of HCV infection (n=150)			
Acute	78	52	
Chronic	15	10	
Treated	57	38	<0.05

***HCV Hepatitis C virus**

2. Natural Killer Cells Profiling

Table 1: Multifaceted Analysis of NK Cell Subpopulations and HCV Patient Characteristics

Characteristic	Overall Mean±SD	Mean±SD (Male)	Mean±SD (Female)
CD56+ NK cells	21±4	22±6	18±4
CD16+ NK cells	9±4	8±2	12±4
CD56^{Bright} CD16^{Dim}/- NK cells	9±4	8±2	12±4
CD56^{Dim} CD16+ NK cells	11±3	14±5	6±2
CD56^{Bright} CD16^{Dim}/- NK cells with comorbidities	6±1	4±1	6±2
CD56^{Dim} CD16+NK cells with comorbidities	7±1	6±2	4±1

***Natural Killer, Cluster of Differentiation, Mean±SD**

3. Viral Load

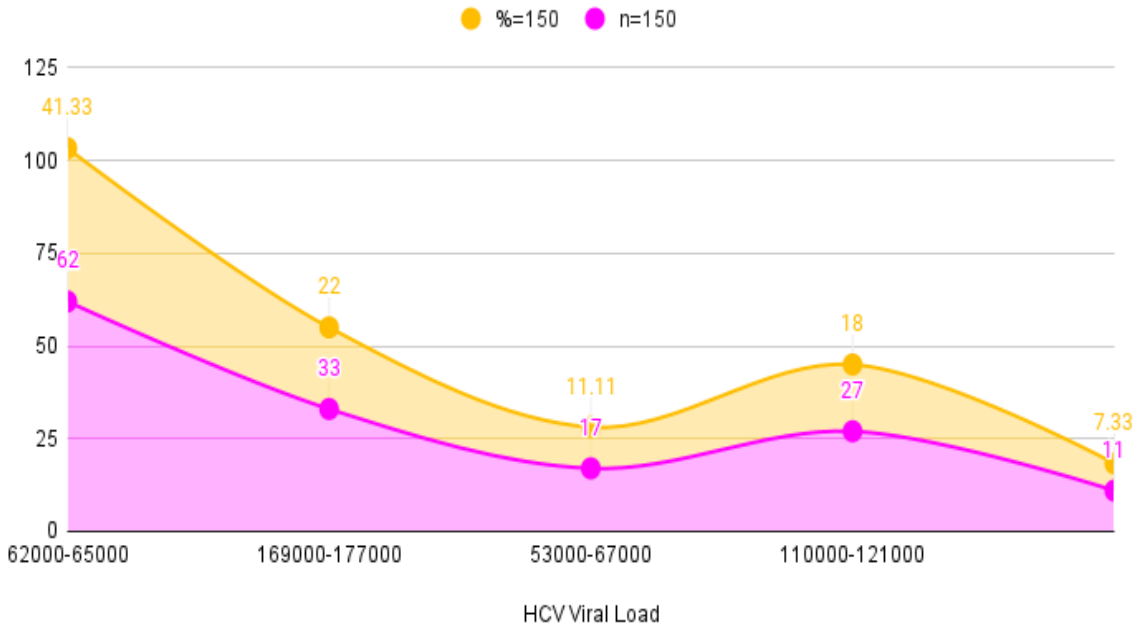

Figure 2: Distribution of HCV Viral Load in the Study Population

4. Liver Biomarkers

Table 3: Evaluation of Liver markers in patients positive with HCV

Liver Markers	Male	Females
ALT	127±8	113±2
AST	131±4	117±1
GGT	62±3	49±2

***ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), GGT (Gamma-Glutamyl Transferase)**

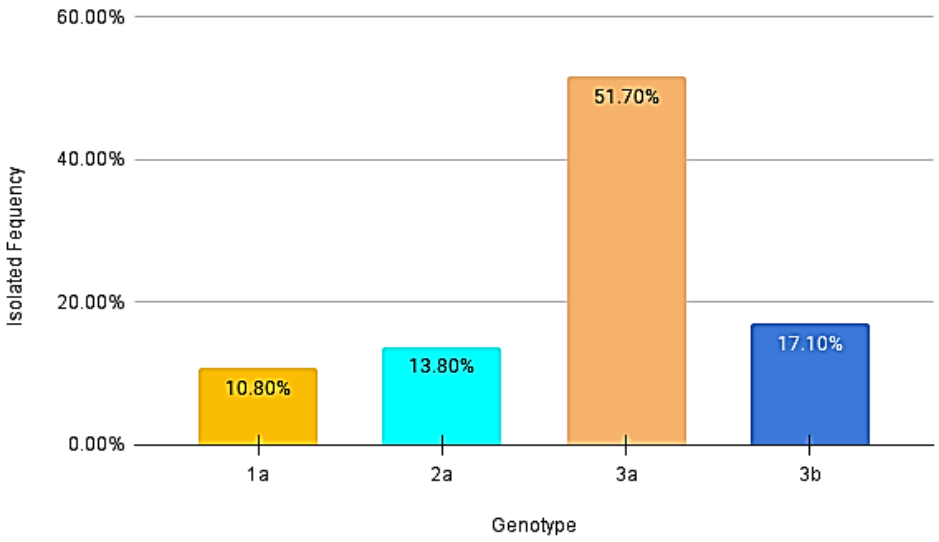

Figure 3: Frequency distribution of Genotypes in patients infected with HCV

Discussion

The table 1 shows the most striking finding was that 87.5% of the patients had liver cirrhosis. This is a serious condition that can lead to liver failure and death. Only 12.5% of the patients had hepatocellular carcinoma, which is a type of liver cancer. 52% of the patients had acute HCV infection, which is a newly acquired infection. 10% of the patients had chronic HCV infection, which is a long-term infection. The remaining 38% of the patients had been treated for HCV infection. There is a significant difference in the percentage of patients with liver cirrhosis between those with acute HCV infection (52%) and those with chronic HCV infection (10%). There is also a significant difference in the percentage of patients with hepatocellular carcinoma between those who have been treated for HCV infection (38%). This suggests that treatment for HCV infection can help to prevent liver cancer.

A study conducted by Bihl et al. revealed that individuals infected with Hepatitis C Virus (HCV) are at a considerably higher risk of developing a condition known as liver cirrhosis when compared to the general population. Liver cirrhosis is a serious medical condition characterized by the scarring of the liver tissue, which can severely impair liver function. The study found that the risk of developing liver cirrhosis was a staggering 22 times greater in people who had HCV infection compared to those without the infection (Bihl et al., 2022).

The overall mean for CD56+ NK cells were 21±4, which means that the average patient had 21±4 percent NK cells per microliter of blood. The mean for males were 22±6 and the mean for females were 18±4. This suggested that males tend to had higher levels of CD56+ NK cells than females. The overall mean for CD16+ NK cells were 9±4, which means that the average patient had 9±4 percent NK cells per microliter of blood. The mean for males was 8±2 and the mean for females were 12±4. This suggested that females tend to had higher levels of CD16+ NK cells than males.

D'Ambrosio et al. studied HCV infection in relation to liver cancer risk. Their study demonstrated that undergoing treatment for HCV infection can lead to a substantial reduction in the risk of developing liver cancer. Specifically, the study indicated that HCV treatment could decrease the likelihood of liver cancer by as much as 70%. This finding underscores the critical role that timely and effective treatment plays in not only managing HCV infection but also in reducing the risk of severe complications such as liver cancer (D'Ambrosio et al., 2022).

The overall mean for CD56^{Bright} CD16^{Dim} NK cells were 9±4, the mean for males were 8±2 and the mean for females were 12±4. This suggested that there is no significant difference in the levels of CD56^{Bright} CD16^{Dim} NK cells between males and females. The overall mean for CD56^{Dim} CD16+ NK

cells were 11±3, the mean for males were 14±5 and the mean for females were 6±2. This suggested that males tend to had higher levels of CD56^{Dim} CD16+ NK cells than females.

The most common viral load range was 62,000-65,000 IU/mL, which accounts for 41.33% of the HCV patients. This followed by the range 169,000-177,000 IU/mL (22%), 53,000-67,000 IU/mL (11.11%), 110,000-121,000 IU/mL (18%), and 310,000-418,000 IU/mL (7.33%). However, there were also a significant number of patients with a high viral load. This suggested that the patients with HCV positive had a variety of severities. The most common genotype is 3a, which is found in 51.70% of the HCV patients. Genotype 2a was found in 13.80%, Genotypes 1a and 3b were found in 10.80% and 17.10%. The different genotypes of HCV can have different clinical implications. Such as genotype 1a was associated with a higher risk of liver cirrhosis and hepatocellular carcinoma than other genotypes (Chuaypen et al., 2022).

The table 3 shows that the mean ALT level for males were 127±8, The mean ALT level for females were 113±2. This suggested that ALT levels were slightly higher in males than females. The mean AST level for males were 131±4, the mean AST level for females were 117±1. This suggested that AST levels were also slightly higher in males than females. The higher levels of ALT and AST can be a sign of liver damage. Men had approximately 10% higher alanine aminotransferase (ALT) levels than women, suggesting a slightly greater tendency for elevated ALT in men. Similarly, revealed that men had around 15% higher aspartate aminotransferase (AST) levels, potentially indicating higher AST in men (Khan, 2022).

The mean GGT level for males were 62±3, the mean GGT level for females were 49±2. This suggested that GGT levels were significantly higher in males than females. GGT levels are more specific for liver damage, but they can also be elevated due to other factors, such as gallstones and certain antiviral medications. Additionally, McLeod et al. indicated that men displayed 30% higher gamma-glutamyl transferase (GGT) levels, implying inherent gender differences in GGT levels. These findings collectively point to gender-related variations in liver enzyme levels, potentially influencing disease markers and health outcomes (McLeod et al., 2022).

Conclusion

The study concluded that liver cirrhosis was the most common condition among patients with HCV infection. This suggested that HCV infection can lead to serious liver damage over time. There was a significant difference in the percentage of patients with liver cirrhosis between those with acute HCV infection (52%) and those with chronic HCV infection (10%). This suggested that chronic HCV infection is more likely to lead to liver cirrhosis. Treatment for HCV infection can help to prevent liver cancer. This supported by the finding that the percentage of patients with hepatocellular carcinoma was lower among those who had been treated for HCV infection (38%). Males tend to have higher levels of CD56+ NK cells and CD56dim CD16+ NK cells than females. This suggested that males may have a stronger immune response to HCV infection. The most common viral load range was 62,000-65,000 IU/mL. This suggested that a significant number of patients with HCV infection had a moderate viral load.

References

1. bihl, F., Bruggmann, P., Castro Batänjer, E., Dufour, J. F., Lavanchy, D., Müllhaupt, B., Negro, F., Razavi, H., Scheidegger, C. & Semela, D. J. L. I. 2022. HCV Disease Burden And Population Segments In Switzerland. 42**,** 330-339.
2. BILBEISI, E., HAMID, A., ABO KHOSA, S. M., TALEB, M. H. & EL AFIFI, A. M. J. F. I. N. 2023. Assessment Of Serum, Dietary Zinc Levels, And Other Risk Factors During The Third Trimester Among Pregnant Women With And Without Pregnancy-Induced Hypertension: A Case-Control Study. 10**,** 1155529.
3. Chuaypen, N., Khlaiphuengsin, A., Prasoppokakorn, T., Susantitaphong, P., Prasithsirikul, W., Avihingsanon, A., Tangkijvanich, P. & Praditpornsilpa, K. J. B. I. D. 2022. Prevalence And

Genotype Distribution Of Hepatitis C Virus Within Hemodialysis Units In Thailand: Role Of HCV Core Antigen In The Assessment Of Viremia. 22**,** 79.

4. D'ambrosio, R., Degasperi, E., Anolli, M. P., Fanetti, I., Borghi, M., Soffredini, R., Iavarone, M., Tosetti, G., Perbellini, R. & Sangiovanni, A. J. J. O. H. 2022. Incidence Of Liver-And Non-Liver-Related Outcomes In Patients With HCV-Cirrhosis After SVR. 76**,** 302-310.
5. Domovitz, T., Ayoub, S., Werbner, M., Alter, J., Izhaki Tavor, L., Yahalom-Ronen, Y., Tikhonov, E., Meirson, T., Maman, Y. & Paran, N. J. M. S. 2022. Hcv Infection Increases The Expression Of ACE2 Receptor, Leading To Enhanced Entry Of Both HCV And SARS-Cov-2 Into Hepatocytes And A Coinfection State. 10**,** E01150-22.
6. Fang, F., Xie, S., Chen, M., Li, Y., Yue, J., Ma, J., Shu, X., He, Y., Xiao, W., Tian, Z. J. C. & Immunology, M. 2022. Advances In NK Cell Production. 19**,** 460-481.
7. Hayes, C. N., Imamura, M., Tanaka, J. & Chayama, K. J. L. I. 2022. Road To Elimination Of HCV: Clinical Challenges In HCV Management. 42**,** 1935-1944.
8. KHAN, A. H. J. P. B. J. 2022. Liver Function Tests In Hepatitis C Patients Of Local Population Of Lahore: Lfts In HEP-C Patients. 12-16.
9. Lucchese, A., Di Stasio, D., Romano, A., Fiori, F., De Felice, G. P., Lajolo, C., Serpico, R., Cecchetti, F. & Petruzzi, M. J. J. O. C. M. 2022. Correlation Between Oral Lichen Planus And Viral Infections Other Than HCV: A Systematic Review. 11**,** 5487.
10. Mcleod, A., Hutchinson, S. J., Weir, A., Barclay, S., Schofield, J., Frew, C. G., Goldberg, D. J., Heydtmann, M., Wilson-Davies, E. J. E. & Infection 2022. Liver Function Tests In Primary Care Provide A Key Opportunity To Diagnose And Engage Patients With Hepatitis C. 150.
11. Shaz, S., Ullah, N. & Rafique, I. J. G. J. C. V. 2019. Prevalence Of Hepatitis B And C In District Dir Upper, Khyber Pakhtunkhwa, Pakistan. 4**,** 8-18.
12. Tembhare, P. R., Chatterjee, G., Chaturvedi, A., Dasgupta, N., Khanka, T., Verma, S., Ghogale, S. G., Deshpande, N., Girase, K. & Sengar, M. J. F. I. O. 2022. Critical Role Of Flow Cytometric Immunophenotyping In The Diagnosis, Subtyping, And Staging Of T-Cell/Nk-Cell Non-Hodgkin's Lymphoma In Real-World Practice: A Study Of 232 Cases From A Tertiary Cancer Center In India. 12**,** 779230.
13. Uzair, B., Abid, S., Saleem, A. & Bibi, K. J. C. C. R. I. Comparison Of Hcv Genotyping Methodologies (Real-Time Pcr And Conventional Pcr). 1322.
14. Wang, W., Zhao, J., Hao, C., Hu, S., Chen, C., Cao, Y., Xu, Z., Guo, J., Xu, L. & Sun, M. J. A. M. 2022. The development of chiral nanoparticles to target NK cells and CD8+ T cells for cancer immunotherapy. 34**,** 2109354.