CLDN6-CAR-T Cell Therapy in Ovarian Cancer: Overcoming the Challenges of Tumour Heterogeneity

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Abstract. Chimeric antigen receptor T cells (CAR-T cells) are synthetically modified versions of T lymphocytes with an additional CAR that allows specific, customised antigen binding and subsequent cell elimination by apoptosis. For ovarian cancer, Claudin-6 (CLDN6) is an excellent target antigen for CAR-T therapy, due to its high expression within ovarian cancer tissue and low expression in healthy adult cells. Scientists have therefore developed CLDN6-specific CAR-T cell therapy (CLDN6-CAR-T). CLDN6-CAR-T offers benefits compared to traditional surgery and chemotherapy, but faces immature clinical outcomes and heterogeneity-related limitations, including antigen escape, TME immunosuppression, and cancer-associated fibroblasts. In the face of these challenges, possible solutions currently being examined and developed include multi-target therapy, CXCR2-targeting, bionic physical barriers, and the use of heparanase.

Keywords: CLDN6-CAR-T; Immunotherapy; Ovarian Cancer; Heterogeneity.

1. Introduction

Ovarian tumours exist in a complex assortment of subtypes [1]. Depending on their specific tissue of origin and stage of development, ovarian tumors are categorized into epithelial ovarian cancers (90% of cases), germ cell ovarian cancers, and sex-cord stromal tumors, with subsequent subcategories, each primarily corresponding to a traditional surgical approach and/or chemotherapy [2].

A relatively new and promising form of immunotherapy developed in recent years, CAR-T cell therapy utilizes the addition of artificially engineered CARs (chimeric antigen receptors) to T lymphocytes, enabling them to bind to a designated target antigen [3]. Following the success and FDA approval of CAR-T use to treat B-cell leukemia in 2017 [4], scientists have turned to CAR-T as a potential solution for other cancers, including ovarian cancer. Among the various candidates, CLDN6 (Claudin-6), a membrane protein that is abundantly expressed on ovarian cancer cells but not in adult tissue, is gradually showing promise through preclinical investigations [5].

However, it remains evident that the therapeutic effect of CAR-T is lower in solid tumour cancers than haematologic cancers, owing to the intercellular, intracellular immunosuppressive environments and especially the heterogeneous nature of solid tumours [6] — the wide range of ovarian cancers, in particular epithelial ovarian cancer with its at least five subtypes [7], reflects this histological heterogeneity. These limitations must be addressed to avoid hindering future development and clinical applications. In this review, we evaluate potential solutions, including multi-target therapy, CXCR2-targeting, bionic physical barriers, and heparanase release, that have been explored to address the issues of ovarian cancer heterogeneity.

2. CLDN6-CAR-T Cell Therapy Overview and Mechanisms

2.1 Ovarian Cancer Traditional Treatment

Commonly, benign ovarian tumours are treated with surgery, and malignant ovarian cancers are treated using a combined course of surgery and chemotherapy [2]. The limitations of surgery and chemotherapy in cancer treatment are well-recognised: surgery generates trauma in surrounding

tissues and cells [8]; minimal residual disease from unremoved deposits can also lead to subsequent re-progression and re-proliferation of cancer [9]. Therefore, surgery is frequently used in conjunction with other therapeutic approaches. Chemotherapy, which involves the administration of chemicals by injection or orally, faces several disadvantages, including low solubility in water, the development of drug resistance (notably through efflux pumps), a lack of chemical specificity towards target cells [10], and cytotoxicity towards healthy body cells [9].

2.2 CAR-T Cell Therapy Overview

Chimeric antigen receptor T cell (CAR-T cell) therapy, a relatively new immunotherapy and adoptive cell therapy that just gained official approval in 2017 [6], involves the specific modification of patient T cells, particularly cytotoxic $CD8^+$ T cells [11]. Specifically manufactured artificial chimeric antigen receptor (CAR) proteins are added to the T cells through viral [12] or non-viral insertion of genetic sequences (especially via electroporation, liposomes, or nanoparticles) [13], which permit the synthesis and expression of the receptor protein after intracellular transcription and translation. The CAR enables the T cells to detect and bind to the complementary antigen on target cells, further allowing induced apoptosis by the secretion of perforin and granzymes [12].

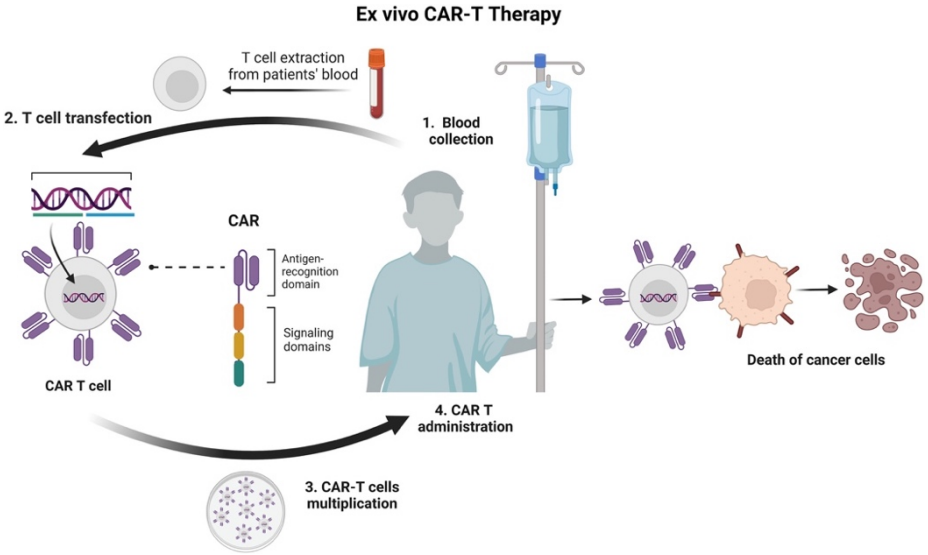


Figure 1. CAR-T cell manufacture and subsequent apoptosis induction in antigen-expressing cancer cells. The CAR gene is inserted into T cells collected by leukapheresis, after which the CAR-T cells are multiplied and infused back into the patient. The CAR structure includes an antigen recognition domain and intracellular signalling domain(s). [14]

A CAR mimics the structure of a natural antibody and typically resembles a linkage of antigen recognition, hinge, transmembrane, and intracellular signaling domains [15]. The antigen recognition domain is a single-chain fragment variant (scFv) of heavy and light regions derived from antibodies, designed to bind with a particular target antigen [12]; the hinge domain enables flexibility of the antigen recognition region; the transmembrane domain provides stability and anchorage to the cell membrane; and the intracellular signalling domain governs cell activation after binding, by setting off necessary signalling pathways within the lymphocyte that enable induction of apoptosis [15].

In contrast to traditional cancer therapy methods, CAR-T cell therapy offers several advantages. For example, unlike surgery and chemotherapy, CAR-T kills cancerous cells with extreme precision, without causing damage to healthy bystander cells. In doing so, as long as they do not express the same targeted antigen [16], the characteristic of engineered lymphocytes being native to the patient [17] also excludes the possibility of immune system rejection. The unique customisability and continual improvability of the CAR structure, along with the non-MHC (major histocompatibility

complex)-reliant mechanism [11], contribute to bolstering the potential of CAR-T as a cancer-solving candidate.

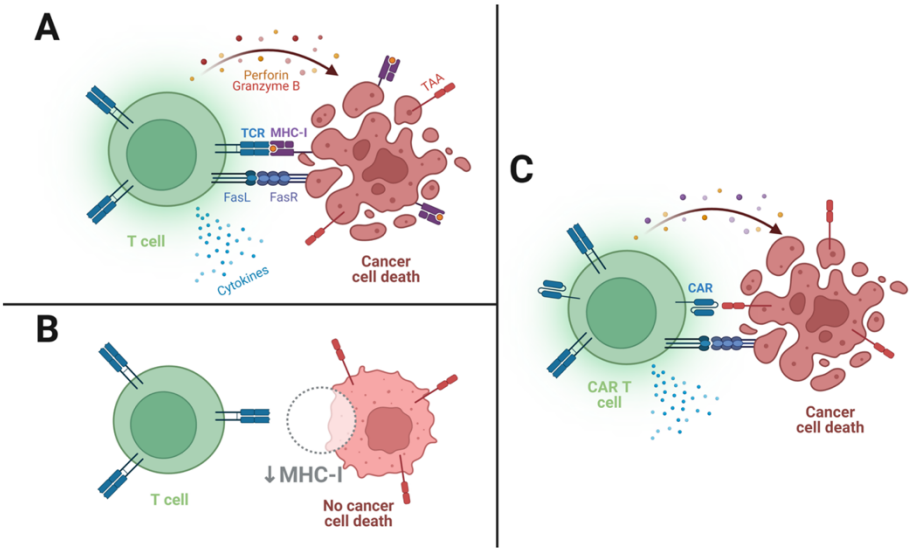


Figure 2. MHC independence of CAR-T cells. (A) Antigen presentation on MHC-I permits target cancer cell elimination by the T cell; (B) MHC-I downregulation inhibits T cell-binding and activation, thus preventing cancer cell elimination; (C) A CAR-modified T cell recognises a tumour-associated antigen and induces cancer cell elimination despite MHC-I downregulation. [18]

2.3 CLDN6 and CAR-T Cell Therapy

Though primarily developed for haematologic cancers, recent preclinical studies have successfully begun to apply CAR-T cell therapy to various solid tumorous cancers, including ovarian cancer. Claudin-6 (CLDN6) is a member of the Claudin (CLDN) family, a group of tight junction transmembrane proteins present on epi and endothelial cells that play a role in paracellular barriers and therefore regulate the permeability of epithelia and endothelia [19], alongside occludin and tricellulin [20].

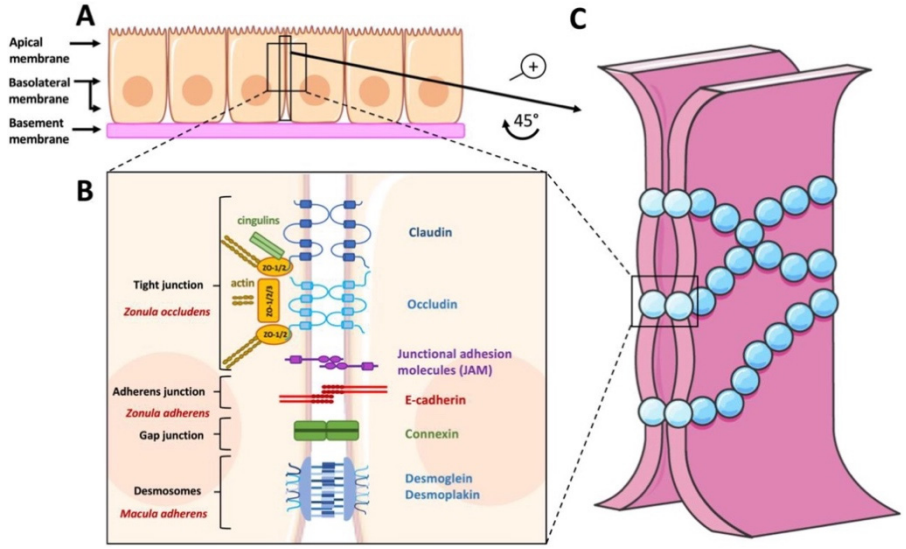


Figure 3. Paracellular junction structures of epithelial cells. (A) Epithelial cells; (B) An enlarged diagram of the tight junction, adherens junction, gap junction, and desmosomes sections between epithelial cells; (C) A three-dimensional representation of the junctions. [21]

CLDN6, which is usually present only in the embryonic tissues of specific organs [20], is overexpressed in ovarian cancers. In contrast, regular adult cells generally lack such expression [22]

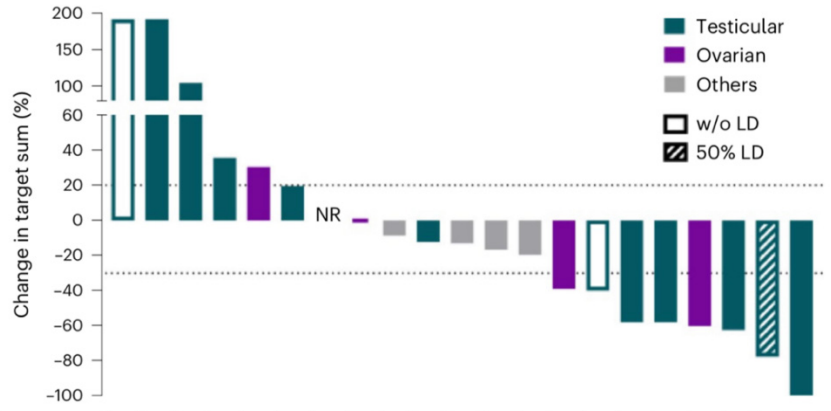
— rendering CLDN6 an ideal oncofetal target for CAR-T therapy [23]. For example, an investigation demonstrated 69.4% of tested ovarian carcinoma samples exhibited CLDN6 expression [24]. It is agreed that CLDN6 is crucial in normal stem cell differentiation and the formation of epithelial tissue, and may be utilized by cancerous cells to increase tight junction leakage for the acquisition of nutrition and growth factors, as well as to create space for invasion and development [25], which explains its high expression in ovarian cancer.

CLDN6-CAR, Claudin 6-specific chimeric antigen receptors, are designed by modifying various domains of the CAR structure. The scFv of the antigen recognition domain that exhibits specificity to CLDN6 is derived from the IMAB206-C46S antibody [26]. The hinge region is made of the CD8α protein, and the intracellular signalling domain below the transmembrane domain consists of a co-stimulatory 4-1BB connected to CD3ζ [27]. CD3ζ contains three portions called immunoreceptor tyrosine-based activation motifs (ITAMs), which undergo phosphorylation upon stimulation and trigger intracellular transduction signalling pathways activating the T cell [28]; 4-1BB, once activated, increases synthesis of the anti-apoptotic proteins bcl-x(L), bfl-1, c-FLIP, and downregulates expression of the ERK-dependent Bim gene, thus preventing activation-induced cell death [29]. Altogether, when a CLDN6-specific CAR-T lymphocyte binds to a CLDN6 molecule, activation and proliferation of the T cell occur, and apoptosis can be induced in the detected CLDN6-expressing cell.

CLDN6-CAR-T offers advantages over other current ovarian cancer CAR-T cell therapies, such as those targeting mesothelin and human epidermal growth factor receptor 2 (HER2) [30]. This is especially due to the minimal to no expression of CLDN6 in healthy adult cells [21], which minimizes the risks of treatment harming normal tissues. Additionally, high expression in cancerous cells remains even after metastasis [31], making CLDN6 an ideal target. However, compared to other therapies, CLDN6-targeting also implies unique precautions related to higher requirements for antibody specificity, due to the structural similarity of CLDN6 to other CLDN proteins present in normal adult cells. CLDN9 especially shares all but three amino acids on its extracellular region and is expressed in skeletal muscle, bone, and brain cells, as well as those in cardiovascular system tissues [31]. Errors in targeting may lead to severe side effects.

3. Limitations in CLDN6-CAR-T Cell Therapy

3.1 Immature Clinical Outcome



CAR-T dose	DL2	DL2	DL1	DL1	DL2	DL2	DL1	DL1	DL2	DL2	DL2	DL2	DL1	DL2	DL2	DL1	DL2	DL2	DL2	DL2	DL2
LD dose (%)	0	100	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	50	100
CARVac	+	+	+	–	–	+	+	–	–	+	–	–	–	+	+	+	–	+	+	+	–
Crossover	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–
Redosing	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	+	+	–	+	–	–
Best response	PD	PD	PD	PD	PD	SD	PD	SD	SD	SD	SD	SD	SD	uPR	PD	cPR	cPR	cPR	uPR	cPR	CR
PD before ACT	NE	+	+	+	+	NE	+	NE	–	–	NE	NE	NE	NE	–	+	–	NE	–	NE	+
PFS, days	46	42	38	46	49	72	29	162	85	NR	87	126	81	79	44	154	119	120	79	289	NR

Figure 4. Clinical data chart and table. 6 patients achieved partial response (PR), 1 achieved complete response (CR), and the ORR was 33%. [27]

In a clinical trial of the CAR-T cell amplifying vaccine (CARVac), it has been demonstrated that a therapy combining the targeting of CLDN6 on ovarian cancer cells with CAR-T technology has a positive effect on cancer treatment. Among the clinical responses of CLDN6-CAR-T infusion ±CARVac, 21 out of 22 patients were treated according to the protocol, including 6 cases of partial response and 1 case of complete response. Therefore, the unconfirmed objective response rate (ORR) was 33% (7/21), and the disease control rate was 67% (14/21). In subsequent repeated evaluations, the total number of observed target lesions continued to decrease [27]. Although the therapy has shown encouraging results, its ORR of less than half indicates that it is still far from maturity.

CLDN6 expression rates in ovarian cancer cells vary due to heterogeneity, which may be considered a significant factor contributing to unsatisfactory clinical outcomes.

3.2 Heterogeneity Affecting the Efficacy of Therapy

Ovarian cancer is heterogeneous (as can be seen from its large number of subtypes). Its expression varies among different individuals or within different cells of the same individual [32]. Differences in tumour microenvironments (TMEs) [33], genetic factors [34], and temporal conditions [35] are important factors contributing to heterogeneity.

A common cause of CAR-T therapy ineffectiveness is antigen escape. Due to heterogeneity, tumour subclonal cells differentiate into antigen-negative and antigen-positive types. Under therapeutic pressure, antigen-negative tumour subclones are selected and proliferated, allowing them to evade attack by losing their antigens, which results in tumour recurrence. At the same time, the remaining antigen-positive cells may continuously stimulate CAR-T cells at low levels, leading to CAR-T cell exhaustion [36]. Such implications of heterogeneous expression are also unavoidable for the CLDN6 protein, despite its relatively high presence in ovarian cancer tumors.

Furthermore, as with other solid tumor-targeting CAR-T therapies, immunosuppression in the TME also reduces the efficacy of CLDN6-CAR-T [37]. In heterogeneous tumour regions, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), M2 macrophages, and tumour-associated macrophages (TAMs) interact either by cell contact or secretion of cytokines such as transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10), hindering CAR-T functionality and activity [38][39]. Moreover, hypoxic regions in metabolic competition, such as the ascites microenvironment in ovarian cancer, or conditions like nutrient deficiency and acidic pH, can inhibit the proliferation and cytokine secretion of CAR-T cells [40][41].

The physical barrier constructed by cancer-associated fibroblasts (CAFs) additionally reduces CAR-T efficacy. CAFs are the main stromal cells in the TME. They secrete a large amount of extracellular matrix (ECM) components, such as collagen and fibronectin, to form a tight physical barrier [42]. This causes difficulty in CAR-T movement and infiltration towards the tumour cells [43].

4. Future Prospects

4.1 Multi-Target Therapy and CLDN6 Targeting Reinforcement

The design of multi-target therapy can effectively alleviate the escape of single-target antigens. Similar to CLDN6, PRAME, and CTCFL are strictly tumour-specific antigens, and their expression in ovarian cancer is more than 20 times greater than in healthy tissues (excluding reproductive organs). PRAME and CTCFL are promising dual targets for CLDN6-CAR-T treatment of ovarian cancer. In the trial, PRAME-specific TCR-T cells demonstrated high cytotoxicity and exhibited significant tumor-suppressive effects against primary ovarian cancer cells, both in vitro and in vivo. CTCFL-directed TCR-T cells also effectively recognized primary patient-derived cells, confirming their antigenic specificity [44]. Jointly targeting these antigens can expand the coverage of heterogeneous ovarian tumours in CLDN6-CAR-T therapy.

Mesothelin (MSLN) is another ideal target that can serve as a dual with CLDN6. Similar to PRAME and CTCFL, MSLN is a cell-surface protein lowly expressed on normal skin cells [45] but

highly expressed in a variety of epithelial-derived cancer tissues, including ovarian cancer [46][47]. This makes MSLN as precise in positioning as CLDN6.

With multiple target sites, even if tumour cells downregulate or lose CLDN6 expression, the targets PRAME, CTCFL, and MSLN can still be recognized by CAR-T, thereby avoiding complete evasion. Currently, there is a lack of mature clinical data on the dual-target or multi-target CAR-T treatment of ovarian cancer targeting CLDN6 and another target; however, this is a promising direction for future development.

In addition to directing focus towards alternative targets, an investigation has also been conducted into other methods of CLDN6-targeting and the consolidation of the CLDN6-CAR-T cell therapy. In a set of preclinical investigations, where CLDN6-CAR-T demonstrated robust and specific tumor regression in mouse models of solid tumor cancers, a nanoparticulate RNA vaccine was designed to augment CLDN6 expression on cells, thereby increasing the persistence of CLDN6-CAR-T in vivo and strengthening the cytotoxic response [48]. In another study, BNT142, an RNA-containing lipid nanoparticle carrying genes for a T cell-engaging bispecific antibody that binds to CD3 markers, was also demonstrated in animal models to generate potent cytotoxic activity against CLDN6-positive tumor cells [49]. Regarding an extension of the T cell concept, remarkable tumor reduction was achieved by an antibody-drug conjugate designated CLDN6-23-ADC in xenograft models expressing CLDN6, indicating an effective method for targeting CLDN6 via non-cellular platforms [22]. The shared capacity of CLDN6-CAR-T and other CLDN6-targeted therapies, as illustrated in these findings, could potentially be integrated to enhance the efficacy of heterogeneous ovarian cancer treatments.

4.2 CXCR2-Targeting and Bionic Physical Barriers

MDSCs play a pivotal role in promoting immunosuppression through multiple pathways within the TME [50]. They secrete immunosuppressive mediators, including inducible nitric oxide synthase (iNOS), arginase-1 (ARG1), and TGF-β, alongside immune-evasive niche-reinforcing IL-10, cyclooxygenase-2 (COX2), and indoleamine 2,3-dioxygenase (IDO) [51][52][53]. In cancers, the G protein-coupled C-X-C motif chemokine receptor 2 (CXCR2) plays a significant role in recruiting MDSCs to the TME [54].

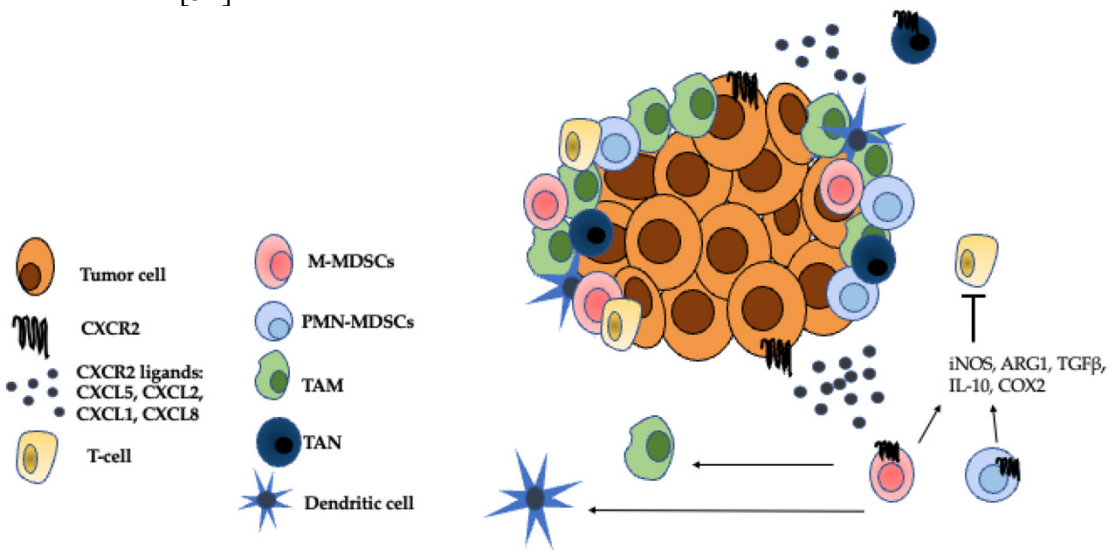


Figure 5. Immunosuppressive cells in the TME. CXCL1, CXCL2, CXCL5, and CXCL8 recruit PMN-MDSCs and M-MDSCs to form an immunosuppressive physical barrier [55]. PNS-MDSCs and M-MDSCs secrete iNOS, ARG1, TGF-β, IL-10, and COX2, inhibiting T cell function [53]. [56]

To enhance T cell infiltration within tumours, scientists are developing CXCR2-modified CAR-T cells [57][58][59]. CXCR2 antagonists are currently being investigated as anti-inflammatory

treatments for various diseases, including chronic obstructive pulmonary disease (COPD), type 1 diabetes, rheumatoid arthritis, ulcerative colitis, and cancer [60]. The CXCR1/2 inhibitor reparixin was found to be safe and well-tolerated in clinical trials for HER-2-negative breast cancer, resulting in a successful reduction in cancer stem cells within patient tumors [61]. In mouse models of head and neck cancer, treatment with the CXCR1/2 inhibitor SX-682 prevented the migration of MDSCs into tumors and enhanced the effectiveness of natural killer cell therapy [62]. The same compound was also later tested in non-small-cell lung cancer models, where dual inhibition of SHP2 and CXCR1/2 successfully reshaped the tumour microenvironment, increased $CD8^+$ T cell activity, and led to slower tumour growth and longer survival [63].

Evidence from liver cancer models linked to non-alcoholic steatohepatitis further supports this approach. In these studies, CXCR2 inhibition reduced the number of pro-tumour neutrophils and enhanced the response to anti-PD1 therapy, producing tumour regression and improved outcomes in mice [64]. Broader reviews have described CXCR2 as a central regulator of neutrophil recruitment and immunosuppression, summarizing growing clinical and preclinical data that support CXCR2 inhibitors, such as SX-682 and navarixin, in solid tumors [65]. However, more recent animal studies have shown that blocking CXCR2 does not completely prevent neutrophil entry into circulation, suggesting that single-agent inhibition may need to be combined with other treatments to achieve lasting immune control [66]. Although no such drugs have yet been approved for treatment, targeting CXCR2 to inhibit MDSC recruitment and counteract the immunosuppressive TME in line with CLDN6-CAR-T cell therapy provides a potentially feasible therapeutic approach to ovarian cancer.

Additionally, bionic physical barriers also offer another unique direction for combating immunosuppression. A research team has designed a bionic physical barrier centred on a thermosensitive hydrogel that can temporarily block the interaction between T cells and tumour tissues. Once T cells accumulate to an optimal level in the tumour tissues, the barrier can be removed by accepting near-infrared light, allowing T cells to attack tumour cells in a more effective state [67]. This therapy reduces the exhaustion of T cells under immunosuppression and represents an innovative approach for tumour immunotherapy that can be applied to CDLN6-CAR-T.

4.3 Use of HPSE to Break Down the ECM

Heparan sulphate (HS) is an important component of the ECM. The enzyme heparanase (HPSE) can degrade HS, thereby breaking the physical obstacle that hinders CAR-T action. Designed HPSE-expressing CAR-T cells (by gene modification) showed enhanced infiltration and tumour suppression in xenograft tumour models from an experiment by Caruana et al. [68]. This work first demonstrated that using HPSE to enhance the ability of immune cells could help overcome the physical obstacles to cell-based immunotherapy in solid cancers.

In subsequent research on other immune cells, a membrane-bound, constitutively active form of HPSE was constructed in natural killer cells. The modified cells maintained continuous enzymatic activity on their surfaces and demonstrated improved tumor infiltration and growth control in animal models [69]. Evidence from immune regulation studies has further supported this approach, where Tregs were found to use HPSE to obtain IL-2 bound to the ECM, enabling them to survive and function within inflamed tissues [70].

Together, these findings support that controlled use of HPSE can enhance tissue penetration and support immune cell function without the need for broad systemic matrix degradation, suggesting a promising route to improve the efficiency of CLDN6-CAR-T in ovarian cancer treatment.

5. Conclusion

CLDN6-CAR-T cell therapy, as demonstrated in preclinical studies, has shown significant improvement in ovarian cancer treatment compared to traditional surgery and chemotherapy, utilizing a targetable trait and presenting wide application prospects. However, due to the inherent characteristics of tumour heterogeneity, tumour-induced immunosuppression, and physical

obstruction of cells in the ECM, a gap still exists between the successful treatments of haematologic malignancies and solid tumours. Therefore, it is necessary to continue improving anti-tumoural mechanisms to create breakthroughs in treatment. The production of T cell-resistant cells under the influence of natural heterogeneous CLDN6 expression and cellular evolution, the release of tumor-assisting, CAR-T-obstructing cytokines, and the presence of an additional ECM barrier assisted by CAFs all contribute to limiting the efficacy of CLDN6-specific CAR-T cell function. To confront these limitations, after passing through long preclinical evaluations, new therapies like dual-targeting (CLDN6/MSLN, CLDN6/PRAME, CLDN6/CTCFL) or multi-targeting CAR-T may be used for patients encountering partial, varying lack of antigen proteins on tumour tissues; other novel strategies, such as CXCR2-targeting to inhibit MDSC recruitment, thermosensitive hydrogel barriers preventing T cell-exhaustion, and using enzymes such as heparanase on the ECM, are also worthy of expansion. These may be implemented into future CLDN6-CAR-T regulation of antitumor activity, with or without combined use with other drugs or therapies. Altogether, further development of CLDN6-CAR-T may provide potential tools not only for combating ovarian cancer, but also for other solid tumors and tumor cancers.

References

[1] A. C. Veneziani et al., "Heterogeneity and treatment landscape of ovarian carcinoma," Nature Reviews Clinical Oncology, vol. 20, no. 12, pp. 820–842, Dec. 2023, doi: https://doi.org/10.1038/s41571-023-00819-1.

[2] Ovarian Cancer Action, "Types of ovarian cancer | Ovarian cancer information," Ovarian Cancer Action, Jun. 03, 2024. https://ovarian.org.uk/ovarian-cancer/types-of-ovarian-cancer/

[3] R. C. Sterner and R. M. Sterner, "CAR-T Cell therapy: Current Limitations and Potential Strategies," Blood Cancer Journal, vol. 11, no. 4, Apr. 2021, doi: https://doi.org/10.1038/s41408-021-00459-7.

[4] R. C. Larson and M. V. Maus, "Recent Advances and Discoveries in the Mechanisms and Functions of CAR T Cells," Nature Reviews Cancer, vol. 21, no. 3, pp. 145–161, Jan. 2021, doi: https:// doi. org/ 10.1038/s41568-020-00323-z.

[5] J. Li et al., "NK-92MI Cells Engineered with Anti-claudin-6 Chimeric Antigen Receptors in Immunotherapy for Ovarian Cancer," International Journal of Biological Sciences, vol. 20, no. 5, pp. 1578–1601, Feb. 2024, doi: https://doi.org/10.7150/ijbs.88539.

[6] National Cancer Institute, "CAR T Cells: Engineering Immune Cells to Treat Cancer," Cancer.gov, Feb. 26, 2025. https://www.cancer.gov/about-cancer/treatment/research/car-t-cells#closing-in-on-car-t-cell-therapy-for-solid-tumors

[7] S. P. Blagden, "Harnessing Pandemonium: The Clinical Implications of Tumor Heterogeneity in Ovarian Cancer," Frontiers in Oncology, vol. 5, Jun. 2015, doi: https://doi.org/10.3389/fonc.2015.00149.

[8] National Cancer Institute, "Surgery," Cancer.gov, Apr. 29, 2015. https://www.cancer.gov/about-cancer/ treatment/ types/surgery

[9] A. Zafar, S. Khatoon, M. J. Khan, J. Abu, and A. Naeem, "Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy," Discover Oncology, vol. 16, no. 1, Apr. 2025, doi: https://doi.org/10.1007/s12672-025-02198-8.

[10] M. Chidambaram, R. Manavalan, and K. Kathiresan, "Nanotherapeutics to Overcome Conventional Cancer Chemotherapy Limitations," Journal of Pharmacy & Pharmaceutical Sciences, vol. 14, no. 1, pp. 67–77, Feb. 2011, doi: https://doi.org/10.18433/J30C7D.

[11] H. E. Hughes-Parry, R. S. Cross, and M. R. Jenkins, "The Evolving Protein Engineering in the Design of Chimeric Antigen Receptor T Cells," International Journal of Molecular Sciences, vol. 21, no. 1, p. 204, Dec. 2019, doi: https://doi.org/10.3390/ijms21010204.

[12] A. Alnefaie et al., "Chimeric Antigen Receptor T-Cells: An Overview of Concepts, Applications, Limitations, and Proposed Solutions," Frontiers in Bioengineering and Biotechnology, vol. 10, p. 797440, Jun. 2022, doi: https://doi.org/10.3389/fbioe.2022.797440.

[13] H. Balke-Want et al., “Non-viral chimeric antigen receptor (CAR) T cells going viral,” Immuno-Oncology and Technology, vol. 18, p. 100375, Jun. 2023, doi: https://doi.org/10.1016/j.iotech.2023.100375.

[14] T. A. Bui, H. Mei, R. Sang, D. G. Ortega, and W. Deng, “Advancements and challenges in developing in vivo CAR T cell therapies for cancer treatment,” EBioMedicine, vol. 106, p. 105266, Aug. 2024, doi: https://doi.org/10.1016/j.ebiom.2024.105266.

[15] U. Ahmad et al., “Chimeric antigen receptor T cell structure, its manufacturing, and related toxicities; A comprehensive review,” Advances in Cancer Biology - Metastasis, vol. 4, p. 100035, Jul. 2022, doi: https://doi.org/10.1016/j.adcanc.2022.100035.

[16] Z. Zhao, Y. Chen, N. M. Francisco, Y. Zhang, and M. Wu, “The application of CAR-T cell therapy in hematological malignancies: advantages and challenges,” Acta Pharmaceutica Sinica B, vol. 8, no. 4, pp. 539–551, Jul. 2018, doi: https://doi.org/10.1016/j.apsb.2018.03.001.

[17] Myeloma UK, “Ask the Nurse: CAR-T cell treatments,” Myeloma UK, Feb. 2022. https://www. myeloma. org.uk/library/ask-the-nurse-car-t-cells/

[18] R. C. De Marco, H. J. Monzo, and P. M. Ojala, “CAR T Cell Therapy: A Versatile Living Drug,” International Journal of Molecular Sciences, vol. 24, no. 7, Mar. 2023, doi: https://doi.org/10.3390/ ijms 24076300.

[19] D. Günzel and A. S. L. Yu, “Claudins and the modulation of tight junction permeability,” Physiological reviews, vol. 93, no. 2, pp. 525–569, 2013, doi: https://doi.org/10.1152/physrev.00019.2012.

[20] G. Krause, L. Winkler, S. L. Mueller, R. F. Haseloff, J. Piontek, and I. E. Blasig, “Structure and function of claudins,” Biochimica et biophysica acta, vol. 1778, no. 3, pp. 631–645, 2007, doi: https://doi.org/ 10. 1016/ j.bbamem.2007.10.018.

[21] A. Berselli, F. Benfenati, L. Maragliano, and G. Alberini, “Multiscale modelling of claudin-based assemblies: A magnifying glass for novel structures of biological interfaces,” Computational and Structural Biotechnology Journal, vol. 20, pp. 5984–6010, Oct. 2022, doi: https://doi.org/ 10.1016/j. csbj. 2022.10.038.

[22] M. S. J. McDermott et al., “Preclinical Efficacy of the Antibody–Drug Conjugate CLDN6–23-ADC for the Treatment of CLDN6-Positive Solid Tumors,” Clinical Cancer Research, vol. 29, no. 11, pp. 2131–2143, 2023, doi: https://doi.org/10.1158/1078-0432.ccr-22-2981.

[23] H. Qu, J. Qiu, and C. Quan, “CLDN6: From Traditional Barrier Function to Emerging Roles in Cancers,” International Journal of Molecular Sciences, vol. 22, no. 24, Dec. 2021, doi: https://doi.org/10. 3390/ijms 222413416.

[24] L. Wang et al., “Clinicopathologic significance of claudin-6, occludin, and matrix metalloproteinases-2 expression in ovarian carcinoma,” Diagnostic Pathology, vol. 8, no. 1, Nov. 2013, doi: https://doi.org/ 10.1186/ 1746-1596-8-190.

[25] H. Du, X. Yang, J. Fan, and X. Du, “Claudin 6: Therapeutic prospects for tumours, and mechanisms of expression and regulation (Review),” Molecular Medicine Reports, vol. 24, no. 3, Jul. 2021, doi: https://doi.org/10.3892/mmr.2021.12316.

[26] L. Seidmann et al., “The Chimeric Antigen Receptor T Cell Target Claudin 6 Is a Marker for Early Organ-Specific Epithelial Progenitors and Is Expressed in Some Pediatric Solid Tumor Entities,” Cancers, vol. 17, no. 6, p. 920, Mar. 2025, doi: https://doi.org/10.3390/cancers17060920.

[27] A. Mackensen et al., “CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial,” Nature Medicine, vol. 29, no. 11, pp. 2844–2853, Oct. 2023, doi: https://doi.org/10.1038/s41591-023-02612-0.

[28] F. Rieux-Laucat et al., “Inherited and Somatic CD3ζ Mutations in a Patient with T-Cell Deficiency,” The New England Journal of Medicine, vol. 354, no. 18, pp. 1913–1921, May 2006, doi: https://doi.org/ 10. 1056/nejmoa053750.

[29] R. Singh, Y.-H. Kim, S.-J. Lee, H.-S. Eom, and B. K. Choi, “4-1BB immunotherapy: advances and hurdles,” Experimental & Molecular Medicine, vol. 56, no. 1, pp. 32–39, Jan. 2024, doi: https://doi.org/ 10.1038/s12276-023-01136-4.

[30] C. Cutri-French, D. Nasioudis, E. George, and J. L. Tanyi, “CAR-T Cell Therapy in Ovarian Cancer: Where Are We Now?,” Diagnostics, vol. 14, no. 8, p. 819, Apr. 2024, doi: https://doi.org/10.3390/ diagnostics14080819.

[31] B. Screnci et al., “Antibody specificity against highly conserved membrane protein Claudin 6 driven by single atomic contact point,” iScience, vol. 25, no. 12, pp. 105665–105665, Nov. 2022, doi: https://doi. org/ 10.1016/j.isci.2022.105665.

[32] D. D. Bowtell et al., “Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer,” Nature Reviews Cancer, vol. 15, no. 11, pp. 668–679, Oct. 2015, doi: https://doi.org/10.1038/ nrc4019.

[33] C. E. Meacham and S. J. Morrison, “Tumour heterogeneity and cancer cell plasticity,” Nature, vol. 501, no. 7467, pp. 328–337, Sep. 2013, doi: https://doi.org/10.1038/nature12624.

[34] M. Gerlinger et al., “Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing,” New England Journal of Medicine, vol. 366, no. 10, pp. 883–892, Mar. 2012, doi: https://doi.org/10.1056/nejmoa1113205.

[35] M. Greaves and C. C. Maley, “Clonal evolution in cancer,” Nature, vol. 481, no. 7381, pp. 306–313, Jan. 2012, doi: https://doi.org/10.1038/nature10762.

[36] D. Gumber and L. D. Wang, “Improving CAR-T immunotherapy: Overcoming the challenges of T cell exhaustion,” eBioMedicine, vol. 77, p. 103941, Mar. 2022, doi: https://doi.org/10. 1016/j.ebiom. 2022. 103941.

[37] M. Ijaz et al., “Overcoming barriers in glioblastoma: The potential of CAR T cell immunotherapy,” Theranostics, vol. 15, no. 14, pp. 7090–7126, Jun. 2025, doi: https://doi.org/10.7150/thno.114257.

[38] K. P. Lim et al., “CD4+CD25hiCD127low Regulatory T Cells Are Increased in Oral Squamous Cell Carcinoma Patients,” PLoS ONE, vol. 9, no. 8, p. e103975, Aug. 2014, doi: https://doi.org/10.1371/ journal. pone.0103975.

[39] Y. Qi, L. Zhang, Y. Liu, Y. Li, Y. Liu, and Z. Zhang, “Targeted modulation of myeloid-derived suppressor cells in the tumor microenvironment: Implications for cancer therapy,” Biomedicine & Pharmacotherapy, vol. 180, p. 117590, 2024, doi: https://doi.org/10.1016/j.biopha.2024.117590.

[40] M. Motallebnezhad, F. Jadidi-Niaragh, E. S. Qamsari, S. Bagheri, T. Gharibi, and M. Yousefi, “The immunobiology of myeloid-derived suppressor cells in cancer,” Tumor biology, vol. 37, no. 2, pp. 1387–1406, Nov. 2015, doi: https://doi.org/10.1007/s13277-015-4477-9.

[41] K. Newick, S. O’Brien, E. Moon, and S. M. Albelda, “CAR T Cell Therapy for Solid Tumors,” Annual Review of Medicine, vol. 68, no. 1, pp. 139–152, Jan. 2017, doi: https://doi.org/10.1146/annurev-med-062315-120245.

[42] M. Schwab, Encyclopedia of Cancer, 4th ed. Springer Berlin, Heidelberg, 2017. doi: https://doi.org/ 10.1007/ 978-3-662-46875-3.

[43] J. M. Pitt, A. Marabelle, A. Eggermont, J.-C. . Soria, G. Kroemer, and L. Zitvogel, “Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy,” Annals of Oncology, vol. 27, no. 8, pp. 1482–1492, 2016, doi: https://doi.org/10.1093/annonc/mdw168.

[44] R. A. van Amerongen et al., “PRAME and CTCFL-reactive TCRs for the treatment of ovarian cancer,” Frontiers in immunology, vol. 14, Mar. 2023, doi: https://doi.org/10.3389/fimmu.2023.1121973.

[45] T. K. Bera and I. Pastan, “Mesothelin Is Not Required for Normal Mouse Development or Reproduction,” Molecular and Cellular Biology, vol. 20, no. 8, pp. 2902–2906, Apr. 2000, doi: https://doi.org/ 10.1128/ mcb.20.8.2902-2906.2000.

[46] M. Schwab, Encyclopedia of Cancer, 3rd ed. Springer Berlin, Heidelberg, 2011. doi: https://doi. org/10. 1007/978-3-642-16483-5.

[47] A. Morello, M. Sadelain, and P. S. Adusumilli, “Mesothelin-Targeted CARs: Driving T Cells to Solid Tumors,” Cancer Discovery, vol. 6, no. 2, pp. 133–146, Oct. 2015, doi: https://doi.org/10.1158/2159-8290. cd-15-0583.

[48] K. Reinhard et al., “An RNA vaccine drives expansion and efficacy of claudin-CAR-T cells against solid tumors,” Science, vol. 367, no. 6476, pp. 446–453, Jan. 2020, doi: https://doi.org/10.1126/ science.aay 5967.

[49] C. R. Stadler et al., “Preclinical efficacy and pharmacokinetics of an RNA-encoded T cell–engaging bispecific antibody targeting human claudin 6,” Science Translational Medicine, vol. 16, no. 748, May 2024, doi: https://doi.org/10.1126/scitranslmed.adl2720.

[50] J.-I. Youn and D. I. Gabrilovich, “The biology of myeloid-derived suppressor cells: The blessing and the curse of morphological and functional heterogeneity,” European Journal of Immunology, vol. 40, no. 11, pp. 2969–2975, 2010, doi: https://doi.org/10.1002/eji.201040895.

[51] O. S. Blomberg, L. Spagnuolo, and K. E. de Visser, “Immune regulation of metastasis: mechanistic insights and therapeutic opportunities,” Disease Models & Mechanisms, vol. 11, no. 10, 2018, doi: https://doi.org/10.1242/dmm.036236.

[52] K. Okła et al., “Clinical Relevance and Immunosuppressive Pattern of Circulating and Infiltrating Subsets of Myeloid-Derived Suppressor Cells (MDSCs) in Epithelial Ovarian Cancer,” Frontiers in Immunology, vol. 10, Apr. 2019, doi: https://doi.org/10.3389/fimmu.2019.00691.

[53] D. I. Gabrilovich, “Myeloid-Derived Suppressor Cells,” Cancer Immunology Research, vol. 5, no. 1, pp. 3–8, 2017, doi: https://doi.org/10.1158/2326-6066.cir-16-0297.

[54] S. Ruixin et al., “CXCR4-modified CAR-T cells suppresses MDSCs recruitment via STAT3/NF-κB/SDF-1α axis to enhance efficacy against pancreatic cancer,” Molecular Therapy, vol. 31, no. 11, pp. 3193–3209, Nov. 2023, doi: https://doi.org/10.1016/j.ymthe.2023.09.010.

[55] D. Raman, P. J. Baugher, Y. M. Thu, and A. Richmond, “Role of chemokines in tumor growth,” Cancer Letters, vol. 256, no. 2, pp. 137–165, 2007, doi: https://doi.org/10.1016/j.canlet.2007.05.013.

[56] K. Bullock and A. Richmond, “Suppressing MDSC Recruitment to the Tumor Microenvironment by Antagonizing CXCR2 to Enhance the Efficacy of Immunotherapy,” Cancers, vol. 13, no. 24, p. 6293, Dec. 2021, doi: https://doi.org/10.3390/cancers13246293.

[57] L. Jin et al., “CXCR1- or CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors,” Nature Communications, vol. 10, no. 1, Sep. 2019, doi: https://doi.org/10.1038/s41467-019-11869-4.

[58] L. M. Whilding et al., “CAR T-Cells Targeting the Integrin αvβ6 and Co-Expressing the Chemokine Receptor CXCR2 Demonstrate Enhanced Homing and Efficacy against Several Solid Malignancies,” Cancers, vol. 11, no. 5, p. 674, May 2019, doi: https://doi.org/10.3390/cancers11050674.

[59] G. Liu et al., “CXCR2-modified CAR-T cells have enhanced trafficking ability that improves treatment of hepatocellular carcinoma,” European Journal of Immunology, vol. 50, no. 5, pp. 712–724, May 2020, doi: https://doi.org/10.1002/eji.201948457.

[60] X. Zhang, R. Guo, H. Kambara, F. Ma, and H. Luo, “The role of CXCR2 in acute inflammatory responses and its antagonists as anti-inflammatory therapeutics,” Current Opinion in Hematology, vol. 26, no. 1, pp. 28–33, 2019, doi: https://doi.org/10.1097/moh.0000000000000476.

[61] L. J. Goldstein et al., “A window-of-opportunity trial of the CXCR1/2 inhibitor reparixin in operable HER-2-negative breast cancer,” Breast cancer research: BCR, vol. 22, no. 1, p. 4, Jan. 2020, doi: https://doi.org/10.1186/s13058-019-1243-8.

[62] S. Greene et al., “Inhibition of MDSC Trafficking with SX-682, a CXCR1/2 Inhibitor, Enhances NK-Cell Immunotherapy in Head and Neck Cancer Models,” Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, vol. 26, no. 6, pp. 1420–1431, Mar. 2020, doi: https://doi.org/10.1158/1078-0432.CCR-19-2625.

[63] K. H. Tang et al., “Combined Inhibition of SHP2 and CXCR1/2 Promotes Antitumor T-cell Response in NSCLC,” Cancer discovery, vol. 12, no. 1, pp. 47–61, Aug. 2021, doi: https://doi.org/10.1158/2159-8290.cd-21-0369.

[64] J. Leslie et al., “CXCR2 inhibition enables NASH-HCC immunotherapy,” Gut, vol. 71, no. 10, pp. 2093–2106, Apr. 2022, doi: https://doi.org/10.1136/gutjnl-2021-326259.

[65] G. Lazennec, K. Rajarathnam, and A. Richmond, “CXCR2 chemokine receptor - a master regulator in cancer and physiology,” Trends in Molecular Medicine, vol. 30, no. 1, pp. 37–55, 2024, doi: https://doi.org/ 10.1016/j.molmed.2023.09.003.

[66] J. W. Kwak et al., “CXCR1/2 antagonism inhibits neutrophil function and not recruitment in cancer,” OncoImmunology, vol. 13, no. 1, Jul. 2024, doi: https://doi.org/10.1080/2162402x.2024.2384674.

[67] Y. Zhang et al., “Controlling T cell-tumor cell interaction with a biomimetic physical barrier for cancer immunotherapy.,” PubMed, vol. 122, no. 28, Jul. 2025, doi: https://doi.org/10.1073/pnas.2500589122.

[68] Caruana et al., “Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes,” Nature Medicine, vol. 21, no. 5, pp. 524–529, May 2015, doi: https://doi.org/10. 1038/nm. 3833.

[69] L.-R. Sofia et al., “An integral membrane constitutively active heparanase enhances the tumor infiltration capability of NK cells,” Oncoimmunology, vol. 14, no. 1, Dec. 2024, doi: https://doi.org/ 10.1080/ 2162402X.2024.2437917.

[70] H. A. Martinez et al., “Regulatory T cells use heparanase to access IL-2 bound to extracellular matrix in inflamed tissue,” Nature communications, vol. 15, no. 1, Feb. 2024, doi: https://doi.org/10.1038/s41467-024-45012-9.