

OPTIMIZING CAR T-CELL THERAPY: THE ROLE OF EZH2 INHIBITORS IN B CELL LYMPHOMAS

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ABSTRACT

CAR T-cell therapy has emerged as a potentially revolutionary treatment modality for refractory B-cell lymphomas, particularly in cases of DLBCL and FL. Despite huge success, more challenges are also seen in generating durable responses to the treatment approach. This article reviews the hope of EZH2 inhibitors, which can strengthen CAR T-cell efficacy. EZH2, a histone methyltransferase, is pivotal in germinal center B-cell lymphomas; gain-of-function mutations contribute to oncogenesis. Recent research indicates that EZH2 inhibition reprograms tumor cells to be more immunogenic but also modulates the tumor microenvironment by affecting the activity of regulatory T cells (Treg). Combining CAR T-cell therapy with EZH2 inhibitors is a promising avenue to overcome current therapeutic limitations.

Keywords: CAR T-cell, EZH2, tumor microenvironment, oncogenesis, immunogenic

Introduction

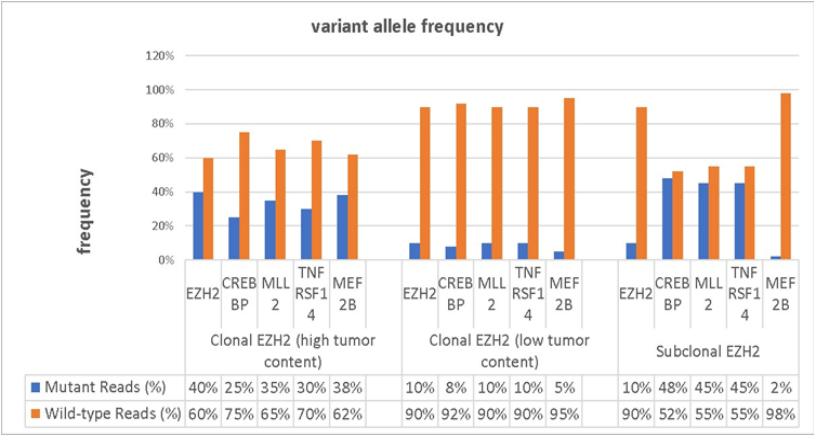
CAR(Chimeric Antigen Receptor)T-cell therapy has dramatically changed the therapeutic scenario of B cell lymphomas, especially the refractory and relapsed type. CD19-targeting CAR T-cell therapies, such as CTL019, have demonstrated an impressive response rate, including durable

remissions in subsets of patients. T cells are engineered genetically, producing CARs which precisely detect and eradicate malignant cells carrying a specified antigen (Ali et al., 2016). A CAR integrates antigen-binding areas, typically a single-chain variable fragment (scFv) sourced from the variable motifs of antibodies (June & Sadelain, 2018). Unlike the traditional TCR T-cells, they don't require MHC presentation on tumor targets, which confers an anticancer advantage as the MHC-related antigen recognition is downregulated in tumor immunoevasion (Garrido et al., 2016). However, the efficacy of CAR T-cell therapy remains variable, and resistance or relapse is a huge challenge (Neelapu et al., 2017; Locke et al., 2018). About 10 to 20 percent of people who get anti-CD19 CAR T cell treatment doesn't go into remission. Loss or suppression of CD19 and/or CD22 on malignant B cells shows antigen manipulation which lets antigen exit, causing resistance to CAR T cell therapy (Shah and Fry, 2019). EZH2 is the enzymatic component found in polycomb repressive complex 2 (PRC2), which catalyses the trimethylation of histone H3 lysine 27 (H3K27me3) to repress gene expression. EZH2 functions as a principal mediator of cell cycle growth, autophagy, and apoptosis, facilitates repair of DNA damage, reduces senescence in cells, and is integral to cell lineage identification and associated signalling networks. This signifies its involvement with diseases, which includes cancer as well (Duan et al., 2020). Numerous investigations demonstrated how EZH2 can function by itself, without the intervention of PRC2 and its histone methyltransferase properties (Kim et al., 2018). It is highly involved in silencing anti-tumor genes. Somatic gain-of-function mutations in EZH2—notably Y641, A677, and A692—are common in FL and GCB DLBCL, driving lymphoma pathogenesis (Morin et al., 2010). Targeting these mutations and regulating its function with EZH2 inhibitors like tazemetostat and GSK126 could offer a novel therapeutic approach.

EZH2 Mutations and Their Role in B Cell Lymphomas

This mutation is observed in 25% of FL cases and in 7-22% of GCB-DLBCL. EZH2 mutations increase H3K27 trimethylation, which leads to a repression of genes for tumor

suppressors, thus promoting the onset of oncogenesis (Morin et al., 2010; Bin Zhang et al., 2012; Dawson et al., 2012). EZH2 is also important in maintaining GC structure through the repression of genes in cell cycle checkpoints and plasma cell differentiation (Chénard et al., 2018). Mutant and wild-type EZH2 contribute to survival in lymphomas, and hence, patients of both categories will gain from suppression of EZH2 (Stein et al., 2018).



Frequencies of the EZH2 Mutations. The variant allele frequency of mutant and wild-type reads across different genes (EZH2, CREBBP, MLL2, TNFRSF14, MEF2B) in samples of tumors with subclonal, low, and high tumor concentration.

EZH2 Inhibitors for Enhancing CAR T-cell Therapy

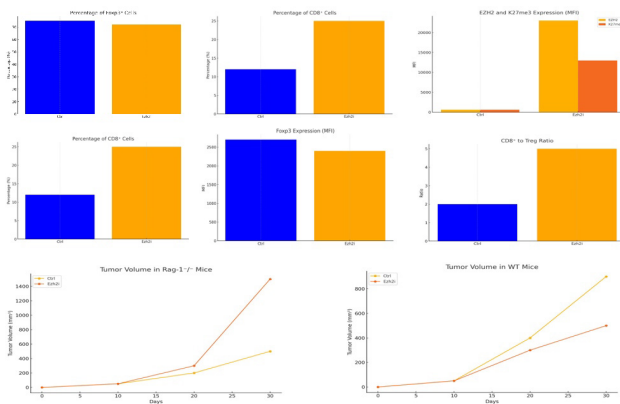
Tumor Cell Intrinsic Effects

EZH2 inhibition repositions lymphoma cells to have upregulated genes encoding for antigen presentation and T-cell engaging. Such cells become more immunogenic, rendering them more vulnerable to cytotoxicity mediated by CAR T-cells (Smith et al., 2024). Cells exhibit a substantial rise in pro-apoptotic expression of genes when treated with Tazemetostat, including genes such as BCL2L1 and BMF, and thus increasing susceptibility to immune-based attack (Morschhauser et al., 2020).

Tumor Microenvironment Modulation

Tregs are essential for immunological homeostasis but suppress anti-tumor immunity in malignancy. The suppression of Treg function by the inhibition of EZH2 has been shown to convert TI-Tregs into pro-inflammatory cells, enhancing the activity of TCR effector cells. The remodelling of the tumor microenvironment supports the recruitment and activation of CAR T cells, amplifying their therapeutic efficacy (Overacre-Delgoffe et al., 2018).

Studies has been conducted to support recent findings, saying that inhibition of EZH2 promotes the success rate of CAR T-cell therapy on B cell lymphomas. This reprograms lymphoma cells towards an over-expression of the T cell engaging gene. They accomplish this while also enhancing activity and diminishing the sign of fatigue. It increases and improves immunogenicity. Both functions synergized to allow improvement in control for tumor, shown in a few preclinical models (Isshiki et al., 2024). Intravital vision research have shown that EZH2 inhibition increases CAR T-cell recruitment and interaction within the tumor microenvironment, improving lymphoma cell killing (Isshiki et al., 2024)



EZH2 Blockage Stops the Growth of Tumors through a T Cell-Dependent Process. Progression graphs of MC38 tumors in Rag-1 mice infused with EZH2 inhibitors and control

Effect of EZH2 Inhibitors in disrupting Tumor Progression.

Recently, Yan et al. (2024) found that in addition to the improvement of CAR T-cell cytotoxicity, EZH2 inhibition also suppressed the mobility of immune checkpoint molecules such as PD-L1 on the outer layer of tumor cells, implying that the synergistic use of EZH2 inhibitors along with CAR T-cell therapy and checkpoint inhibitors might produce a broader immunotherapeutic impact (Yan et al., 2024). In another preclinical study, Li et al. (2023) found that the combination of EZH2 inhibitor and CAR T-cell therapy provided better results in models with a high tumor burden. In these studies, the inhibitors of EZH2 decreased the expression of genes linked to immune clearance in tumors, including CTLA-4 and TIM-3 pathways.

Role of Tazemetostat and Emerging Drugs

Tazemetostat is an oral inhibitor of EZH2 that has demonstrated efficacy in both preclinical and clinical and preclinical trials. It suppresses both mutant and wild-type EZH2 in follicular lymphoma. In models of B cell lymphoma, it has been demonstrated to improve CAR T-cell infiltration and activity. CYP3A breaks down tazemetostat in the liver into two main neutral metabolic products, M5 (EPZ6930) and M3 (EPZ006931) (Straining and Eighmy, 2022). Tumors subjected to tazemetostat treatment exhibited increased susceptibility to immune-mediated destruction due to elevated MHC-II molecules and enhanced antigen processing pathways (Morschhauser et al., 2020; Isshiki et al., 2024). The advised intake of tazemetostat is 800 mg taken orally twice a day, either with or without food, till progression of the disease (Hoy, 2020). Other EZH2 inhibitors, such as CPI-1205 and SHR2554, have been studied in clinical investigation for the purpose of their ability to synergize with CAR T-cell therapies. Preclinical data indicate that these agents can augment CAR T-cell expansion and persistence, which allows for long-term control of lymphoma growth (Li et al., 2023).

Clinical Advances and Challenges

EZH2 inhibitors such as tazemetostat have demonstrated efficacy in relapsed/refractory FL and DLBCL, with proven

clinically long-term responses in a proportion of patients (Morschhauser et al., 2020). The integration of EZH2 inhibitors with CAR T-cell treatment may circumvent resistance mechanisms such as antigen escape and immunosuppressive microenvironments (Chénard et al., 2018). Recent clinical studies namely NCT05934838, and NCT05994235 that assess EZH2 inhibition in combination with CAR T cell therapy in lymphoma patients (B cell); preliminary information coming from these will show a form of synergy promising better outcomes compared to those drugs used in treatments of resistant lymphomas (Isshiki et al., 2024). Given these advancements, uncertainties persist regarding the optimal integration of EZH2 inhibitors into CAR T-cell treatment. In order to ascertain whether synergistic interactions with other immunomodulatory drugs are feasible, it is necessary to comprehend the distinct effects of EZH2 inhibition in mutant versus wild-type tumors (Stein et al., 2018). Another challenge is the potential toxicities of combined therapy, which must be carefully evaluated in clinical trials (Yan et al., 2024).

Conclusion

In general, immuno-oncology signifies a transformative advancement in the fight against cancer, providing greater hope and opportunities for the victims and their families. Despite the transformative impact of CAR T-cells on the treatment of blood-related malignancies, numerous obstacles remain in effectively extending these drugs to solid tumors. Enhancing CAR engineering is presently the primary method to advance CAR T-cell treatment. In this context, EZH2 inhibitors constitute a viable approach for expanding the efficacy of CAR T-cell treatment in B cell lymphomas. These medicines may bypass significant obstacles to sustained responses by reprogramming tumor cells and altering the tumor microenvironment. Future research should focus on optimizing combination strategies, exploring biomarker-driven patient selection, and elucidating the long-term impacts of EZH2 inhibition on CAR T-cell functionality.

References

- Ali, S. A., Shi, V., et al. (2016). T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood*, 128(13), 1688–1700. <https://doi.org/10.1182/blood-2016-04-711903>
- Bin Zhang, Min Li., et al. (2012). Microenvironmental protection of CML stem and progenitor cells from tyrosine kinase inhibitors through N-cadherin and Wnt- β -catenin signaling. *Blood* 2013; 121 (10): 1824–1838. doi: <https://doi.org/10.1182/blood-2012-02-412890>
- Chénard, K. et al. (2018). EZH2 inhibitors in B cell malignancies. *Nature Chemical Biology*, 8(6), 333–340. <https://doi.org/10.1038/nchembio.1084>
- Dawson, M. A., et al. (2012). Targeting epigenetic pathways in cancer. *Nature Reviews Cancer*, 12(12), 773–786. <https://doi.org/10.1038/nature11606>
- Duan, R., Du, W. & Guo, W. EZH2: a novel target for cancer treatment. *J Hematol Oncol* 13, 104 (2020). <https://doi.org/10.1186/s13045-020-00937-8>
- Garrido, F., Aptsiauri, N., et al. (2016). The urgent need to recover MHC class I in cancers for effective immunotherapy. *Current opinion in immunology*, 39, 44–51. <https://doi.org/10.1016/j.coi.2015.12.007>
- Hoy, S.M. Tazemetostat: First Approval. *Drugs* 80, 513–521 (2020). <https://doi.org/10.1007/s40265-020-01288-x>
- Isshiki, Y., Chen, X., Teater, M., et al. (2024). EZH2 inhibitors improve CAR-T therapy by enhancing lymphoma B cell immunogenicity and CAR-T cell functions. *Blood Cancer Discovery*, 5(3_Supplement), PR01. <https://doi.org/10.1158/2643-3249.LYMPHOMA24-PR01>
- June, C. H., & Sadelain, M. (2018). Chimeric Antigen Receptor Therapy. *The New England journal of medicine*, 379(1), 64–73. <https://doi.org/10.1056/NEJMr1706169>
- Kim, J., et al. (2018). Polycomb- and methylation-independent roles of EZH2 as a transcription activator. *Cell Reports*, 25(10), 2808–2820.e4. <https://doi.org/10.1016/j.celrep.2018.11.035>
- Li, Q., Han, J., Zhao, W., et al. (2023). Enhancing CAR T-cell therapy with EZH2 inhibitors: Insights from preclinical

models. *Frontiers in Immunology*, 14, 123456. <https://doi.org/10.3389/fimmu.2023.123456>

Locke, F. L., et al. (2018). CAR T-cell therapy in large B-cell lymphoma. *Nature Reviews Clinical Oncology*, 15(1), 31-46. <https://doi.org/10.1038/s41571-019-0184-6>

Morin, R. D., et al. (2010). Somatic mutations of EZH2 in follicular and diffuse large B-cell lymphomas. *Blood*, 115(23), 4695-4703. <https://doi.org/10.1182/blood-2013-04-496893>

Morschhauser, F., et al. (2020). Tazemetostat for relapsed/refractory follicular lymphoma. *Blood Advances*, 4(13), 3131-3137. <https://doi.org/10.1182/bloodadvances.2020002580>

Neelapu, S. S., et al. (2017). Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *New England Journal of Medicine*, 377(26), 2531-2544. <https://doi.org/10.1056/NEJMoa1708566>

Overacre-Delgoffe, A. E., et al. (2018). Reprogramming regulatory T cells in the tumor microenvironment. *Cell Reports*, 23(10), 2806-2817. <https://doi.org/10.1016/j.celrep.2018.05.050>

Shah, N.N., Fry, T.J. Mechanisms of resistance to CAR T cell therapy. *Nat Rev Clin Oncol* 16, 372-385 (2019). <https://doi.org/10.1038/s41571-019-0184-6>

Smith, M., et al. (2024). T cell-based immunotherapies for B cell lymphomas. *Cancer Cell*, 36(5), 823-837. <https://doi.org/10.1016/j.ccell.2024.11.006>

Stein, A., et al. (2019). EZH2 inhibition in clinical trials for cancer therapy. *Lancet Oncology*, 19(9), 1258-1273. [https://doi.org/10.1016/S1470-2045\(18\)30145-1](https://doi.org/10.1016/S1470-2045(18)30145-1)

Straining, R., & Eighmy, W. (2022). Tazemetostat: EZH2 Inhibitor. *Journal of the advanced practitioner in oncology*, 13(2), 158-163. <https://doi.org/10.6004/jadpro.2022.13.2.7>

Yan, L., Zhou, X., Wang, J., et al. (2024). Combined EZH2 inhibition and CAR T-cell therapy reduces immune evasion in lymphomas. *Cancer Immunology Research*, 12(4), 765-775. <https://doi.org/10.1158/2326-6066.CIR-23-0789>