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CAR-T Cell Therapy in Primary Mediastinal B-cell Lymphoma

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Abstract:

A specific type of CAR-T cell therapy, the CAR19 therapy has demonstrated notable efficacy for relapsed or refractory (R/R) hematologic cancers, including B-cell lymphomas. After the recent reclassification of the rare subtype Primary Mediastinal B-cell Lymphoma (PMBCL) from general Diffuse Large B-cell Lymphoma (DLBCL), questions have been raised about the application of immunotherapies in this cancer, including the most discussed CAR-T cell therapy. The CAR-T cell therapy, more specifically the CAR19, exerts anti-tumor effects in multiple mechanisms, including different cytotoxic pathways—apoptosis triggering via perforin–granzyme release and Fas/FasL-pathway—driven by calcium-dependent signaling MAPK, PI3K/Akt, and NF-κB. Multiple extant clinical trials have evaluated the response of PMBCL patients to CAR19, specifically the Axi-cel, Liso-cel, and Tisa-cel therapies, focusing the rates of complete response (CR), progression-free survival (PFS), and the overall survival (OS). Studies exclusive to PMBCL patients showed that bridging therapies and other first-line treatments did not influence CAR19 effectiveness, while post-relapse application of Pembrolizumab yielded durable CRs in some patients, and allo-HSCT was associated with high mortality. The CAR-SIE trial in Italy, comparing Axi-cel CAR19 in PMBCL cancer with other B-cell lymphomas, demonstrated a better result in PMBCL patients with higher ORR, CR, and PFS. This paper integrates the underlying mechanisms of CAR19 with clinical trials, aiming to demonstrate both the potential and the limitations of this therapy in PMBCL cancer.

Keywords: CAR-T Cell Therapy; CAR19; Primary Mediastinal B-cell Lymphoma.

1. Introduction

Primary Mediastinal B cell lymphoma, abbreviated to PMBCL, is an aggressive subtype of Non-Hodgkin Lymphoma (NHL). It accounts for about 2-3% of all NHLs, mostly affecting adolescents and adults, with a female predominance and an age range of 30-39 [1]. The tumor originates in the mediastinum, the middle space within the thoracic cavity that contains the heart and pericardium.

Previously categorized as part of the cancer subtype DLBCL before it's reclassified as a distinct cancer type, PMBCLs are characterized by the abnormal proliferation of medium and large-sized B cells in the mediastinal lymphoid tissues, alongside with the development of sclerosis. Histologically, the tumor mass is relatively large, visualized in clinical practices through CT (Computed Tomography), PET (Positron Emission Tomography), and MRI (Magnetic Resonance Imaging). Phenotyping of the cancerous B-cells after biopsy indicates the expression of pan-B-cell antigens, including CD19, CD22, CD79a, and CD45, absence of cell surface protein immunoglobulins, and over-expression of receptor CD30, ligands PD-L1/2 of programmed cell death associated with events of 9p24.1 gene translocation and amplification [1].

PMBCLs often require intense treatment programs similar to those of DLBCL cancers, including combination chemoimmunotherapy regimens R-EPOCH and R-CHOP or DA-EPOCH-R [1]. However, treatments for R/R (relapse or refractory) PMBCLs most frequently involve the injection of monoclonal antibodies pembrolizumab (Keytruda), or CAR-T cell therapies Axi-cel (Yescarta) and Liso-cel (Breyanzi), and Tisa-cel (Kymriah). This paper will examine CAR-T cell (CAR19) therapy's mechanisms and its efficacy in PMBCL cancer, alongside with looking upon its combination with different prior and salvage therapies.

2. CAR19 therapy in PMBCL

Initial chemoimmunotherapies are capable of treating 80-85% patients of the cancer, but roughly 20% of patients who are diagnosed later with R/R PMBCL–whether the first-line treatment manifests ineffectiveness or the remission period wasn't desirably sustainable–receive a dismal prognosis [2]. The conventional methodology to tackle R/R PMBCLs has been salvage chemotherapies, autologous stem cell transplantation (ASCT), and checkpoint inhibitor (ICI) therapies. FDA-approved checkpoint inhibitor Pembrolizumab, a PD-1 receptor inhibitor, showed only an average response rate of 48% in patients with relapsed PMBCLs. Over roughly half of the patients who do not respond to salvage therapies, CAR19 cell therapy may be the remedy chosen. CAR19 therapy has CARs' scFv regions designated to recognize the CD19 protein, a pan-B-cell antigen expressed in nearly all malignant B cells. This therapy has demonstrated promising effects in aggressive NHLs, and although only a proportion of patients included were diagnosed with PMBCL in those pivotal studies, the expectation was that there would be minimal discrepancy between PMBCL patients and other DLBCL patients treated with CAR19 T-cell therapy.

3. Mechanisms of CAR19 in Cancer Treatment

Known for its success in hematologic cancers, CAR19 therapy is among the most effective types of Adoptive Cell Therapy (ACT), an immunotherapy leveraged to treat metastasizing or solid tumors via the body's ameliorated immune system. In treating NHL and PMBCL cancers, the CAR19 cell therapy relies on synthetic Chimeric Antigen Receptors (CARs) that recognize the CD19 protein on B cells, where viral vectors will be used to engineer patients' (autologous) or donors' (allogeneic) T cells to express these receptors. CARs are genetically engineered and consist of four key parts: an extracellular antigen binding domain (with scFv region from an antibody) and a hinge region that links and anchors the receptor to the transmembrane and intracellular signaling domains [3]. It is important to note that CAR19 therapy has shown phenomenal clinical results in hematologic cancers, in particular B-cell lymphomas.

Researchers have worked extensively to improve the efficiency and ameliorate the safety risks of CAR-T cell therapy, developing five generations of CARs distinguished predominantly by their different intracellular signaling domains. The therapy 2nd generation of CARs is most used in medical applications, with multiple FDA-approved commercial products such as Idecabtagene vicleucel (Abecma) and Axicabtagene ciloleucel (Yescarta). The intracellular components of the second-generation Chimeric Antigen Receptor include the CD3ζ Chain that provides the primary activation signal, and co-stimulatory domains such as 4-1BB and CD28. Meanwhile, the 4th and 5th generation CARs are still in phase 1 clinical trials.

4. Mechanisms of CAR19

Although engineered CARs and the TCR/CD3 complex contain the same intracellular domain CD3ζ, which promotes signal transduction cascades, the extracellular domain of CARs resembles multiple T cell receptors, including the TCR$\alpha\beta$, CD3$\zeta\zeta$, CD3$\varepsilon\delta$, and CD3$\varepsilon\gamma$ [4]. However, the scFv extracellular domain of the CARs rec-

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ognizes the specific epitope of TAAs (tumor-associated antigens) on the surface of cancerous cells–in the case of PMBCL, the CD19 protein. This process is independent of the need for MHC-Antigen complex presentation by an APC cell. The binding of the ligand to the extracellular receptor domain of CARs triggers a conformational change in the protein, inducing the activation of Immunoreceptor Tyrosine-Based Activation Motifs (ITAMs) in the initiator CD3ζ chain [3].

Forming an immune synapse when CARs bind to the specific antigen, these artificially manufactured receptors trigger similar downstream signaling cascades as un-modified TCRs. However, different signal transduction pathways are triggered at slightly different times due to their structural discrepancies [3]. To elaborate, Src-family kinases Lck and Fyn phosphorylate the CAR's CD3ζ ITAM tyrosines. Subsequently, the biphosphorylated ITAMs, which exist abundantly in zeta chains of CD3, recruit and bind to Zap-70 (Zeta-chain-associated protein kinase 70). The phosphorylation cascade continues to involve protein kinases, phosphorylating linker protein LAT, which activates phospholipase enzymes PLCγ and GRB2. Subsequently, activated enzyme PLCγ then catalyzes the production of secondary messengers diacyglycerol and inositol trisphosphate (IP3) from phosphatidylinositol bisphosphate (PIP2). These secondary messengers stimulate the influx of second messenger Ca^2, and the genetic expression of transcription factors NF-κB, NFAT and more. Therefore, upregulating gene expression of proteins that facilitate T-cell proliferation, cytokine expression, and inflammation [5].

Fundamentally, the signaling effect of CARs consists of of calcium independent pathway Fas/FasL, and calcium dependent pathways: The MAPK pathway, stimulating T-cell proliferation and cytokine production; the PI3K pathway, promoting T-cell survival and metabolism by activating protein Akt, mTOR, and FOXO1,and the NF-κB pathway, which is a transcription factor that supports immune response [6]. Activated CAR-T cells and their intracellular signaling pathways perform their anti-cancer effect via two dominant mechanisms. First, the activated T cells stimulate apoptosis in the malignant B cells via the secretion of lytic granules. These granules consist of perforin, which punctures malignant cell surfaces to allow the entrance of granzymes, a protease in Tc cells and NK cells that enter tumor cells through surface pores and induces apoptosis [3]. Specifically, the granzymes migrate into the plasmalemma of the center supramolecular activation cluster (cSMAC), which clusters of molecules resembling concentric rings within the TCR-initiated Immune Synapse. In the cSMAC, initial response is enhanced by the accumulation of signals, delivering the cytotoxic granules consisting of granzymes into the targeted cell. The subset of MAP-Kinases aids this process: JNK and p38 isoforms upregulate the expression of pro-apoptotic genes and downregulate the transcription of anti-apoptotic genes, and extracellular signal-regulated kinase ERK1/2 redistributes perforin and granzyme A/B to the tumor contact zone [6]. This major 'killing' mechanism involved in CAR-T cell therapy is facilitated by the increase in calcium ion concentration.

The second pathway, in comparison, doesn't require the influx of calcium and is therefore calcium-independent. Those modified cytotoxic T-lymphocytes can trigger apoptosis of target cells via the Fas/FasL pathway, where the Fas 'death receptor', or the TNFRSF6 protein of the target cell, binds to the Fas ligand (FasL) of the T cell, initiating caspase cascades. The canonical NF-κB, including the transcription factors p65 or RelA, promotes gene expression of the Fas protein by binding to the FAS promotor [5]. Then, the Fas ligand and Fas cell death receptor binding will induce high cytotoxicity in the cellular environment, consequently triggering apoptosis, or programmed cell death. The FasL homotrimer achieves this mechanism by the forming a DISC (death-induced signaling complex) after recruiting Fas-associated protein of a Death Domain (FADD), then activating Caspase-8 enzymes. The activated Caspase-8 activates a caspase cascade, which will eventually lead to the formation of a mature caspase, an enzyme that cleaves cellular substrates. CAR receptor T cells also release cytokines such as GM-CSF, IFN-γ and TNF-α, which alter the tumor microenvironment and enhance immune cell's anti-tumor activities [5].

5. Clinical trials of CAR19 in PMBCL

France's national DESCAR-T registry (CARTHYM) analyzed R/R PMBCL outcomes with multiple products of anti-CD19 CAR T, showing promising results most conspicuous for Axi-cel therapy: In the subset of 62 patients receiving Axi-cel, the CR rate was 74.5%, the PFS of two years was 70.4%, and the OS of two years was 86.9% [7]. In this study, around 86.6% of patients received bridging therapy in between CAR19 infusion and leukapheresis, including various polychemotherapies such as EPOCH; around 60.6% received salvage checkpoint inhibitor therapies, pembrolizumab or nivolumab; and 19.6% received radiotherapy. The study also pointed out that a report of good metabolic response in the first month positron emission tomography (PET) scan (Deauville score 1–4/ ΔSUVmax reduction>24%) was associated with long-term recovery in the future. Moreover, results showed that bridging therapies and checkpoint inhibitors, which were thought to have the effect of enhancing T cell activity, did

not affect CAR-T efficacy [7]. Considering the reported clinical trials, CAR19 cell therapy is a valid standard care for relapsed PMBCLs.

A clinical study in the Sheba Medical Center of Israel observed the effect of salvage CAR19 therapy in R/R PMBCL adults, with the prerequisite of at least 2 prior treatment lines in each of the patients. Out of the 20 persons sample size, about 5 (20%) of the patients had a Karnofsky Performance Status (KPS) of less than 90%, 9 (45%) had PMBCL cancer stage of III-IV, and 6 (30%) had a bulky tumor. Evaluation at the first month interval reported ORR of 75%, with CR of 35%. 2 (40%) of the patients with only a partial response achieved a complete response after the implementation of Radiotherapy, 1 patient achieved a CR after allogenic hematopoietic stem cell transplantation. Extended tracking of the patient's status reported 40% of 1-year progression-free survival, and 1-year overall survival of 69%. At the end, the CAR19 therapy was tolerated by around half of the patients, while the death in 10 patients (50%) was caused by progression disease (PD), the uncontrolled metastasis of cancer. Moreover, in the patients that had relapse/progression after 1 year after the reception of CAR-T therapy, 2 (40%) of the patients achieved CR and PFS within three years of monitoring after receiving pembrolizumab; 5 (83%) of the patients who received allo-HSCT deceased within the interval of 1 year. Prembolizumab in combination with CAR19 therapy may be a valid medical care for PMBCL patients, and caution when assigning salvage allo-HSCT is required [8].

6. CAR19 Therapies in NHL Clinical Trials

Due to the rarity of PMBCL, limited clinical trials are recorded, with even fewer dedicated exclusively to patients of this specific NHL. However, CAR-T cell therapy has been adopted as one of the standard salvage therapies for R/R LBCL beyond second treatment line, with PMBCL included within. In the following analysis, clinical data on both widely LBCL (PMBCL included) and exclusively PMBCL will be examined; treatments used within those clinical trials usually utilize 3 commercial products: Axi-cel Yescarta (the first approved CAR-T cell for PMBCL cases), Tisa-cel Kymriah and Liso-cel Breyanzi. Each commercial product has distinct characteristics, manufacturing processes, and reported clinical results.

Axi-cel uses a CD28 co-stimulatory domain in its CARs structure, and is known for rapid T-cell expansion, granting it a relatively short manufacturing time (~14 days, proclaimed by recent developments in the U.S.). This ability is pivotal for rapidly progressing PMBCL patients who cannot wait a long time to receive treatment. In the ZUMA-1 trial, axi-cel achieved high objective response rates ORR in refractory LBCL (~83%). However, axi-cel's CD28 co-stimulation is associated with higher rates of CRS and ICANS.On the other hand, the other product Tisa-cel uses a 4-1BB co-stimulatory domain and has longer manufacturing time. This therapy was initially approved for ALL in children,while later qualified for DLBCL treatment in adults. Tisa-cel's pivotal trial JULIET declared an ORR of ~52% in relapsed DLBCL. The third product, Liso-cel, also uses a 4-1BB domain, but its manufacturing process yields a similar ratio of CD4+ and CD8+ manufactured T cells. It was shown in the TRANSCEND trial to be highly effective in R/R LBCL, with an ORR of 73% and the lowest CRS response of ~42%.

The Italian CAR-SIE observation study, a comparative review of Axi-cel treatment for patients with R/R PMBCL (n=70) versus other LBCL (n=190) has shown prominently better outcomes for PMBCL patients, with a higher survival rate and response rate. A 3-month evaluation showed an objective response rate (ORR) of 69% with a complete response (CR) rate of 65% in PMBCL, whereas results for LBCL were 54% for ORR and 47% for CR. At the end of 12 months, data from the trials showed a progression-free survival (PFS) of 62% in PMBCL versus a PFS of 48% in LBCL. Furthermore, overall survival (OS) was 86% in PMBCL while 71% in LBCL [2]. The results above showed great efficacy of CAR-T cell therapy in PMBCL treatments, which extrapolations may assume that PMBCL patients generally respond better to CAR19 cell therapy compared with other NHL patients.

7. Conclusion

CAR19 T-cell therapy has played an essential role in the treatment of R/R PMBCL cancers as well as other B-cell Lymphomas, with clinical data reporting encouraging response rates and sustainable remissions in patients with otherwise limited therapeutic options–in particular, responses to Axi-cel therapy. Observational clinical trials, such as the DESCAR-T registry, Sheba Medical Center Retrospective analysis, and the CAR-SIE, confirm the promising ORR of patients towards this therapy. but also underscore the limitations of CAR19 effectiveness in R/R PMBCLs in achieving long-term PFS. Studies also highlight the combination of CAR19 therapy with other first-line treatment and salvage therapies upon CAR-T cell failure, with remarkable results shown for the assignment of PD-1 inhibitor Prembolizumab. However, considering the limited sample size, more pivotal trials with a broader sample size need to be conducted to improve the

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understanding of this immunotherapy therapy in PMBCL cancers.The future prospects of CAR19 therapy lie not only in the therapy design and minimizing toxicities, but also in developing rational combination strategies and post-relapse interventions that can achieve a higher rate of progression-free survival.

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