

## Case Report

**Checkpoint Inhibition Followed by CAR-T Cell Therapy in Refractory PMLBCL: Immunologic Response and Fertility Preservation in a Young Patient**

Filomena Emanuela Laddaga <sup>1</sup>, Tommasina Perrone <sup>2</sup>, Pellegrino Musto <sup>2,3</sup>, Ilaria Farella <sup>4</sup>, Stefano Martinotti <sup>4</sup>, Francesco Gaudio <sup>4,\*</sup>

1. Hematology and Cell Therapy Unit, IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; E-Mail: [ematologiauniba@gmail.com](mailto:ematologiauniba@gmail.com)
2. Hematology and Stem Cell Transplantation Unit, AOU Consorziale Policlinico, Bari, Italy; E-Mails: [tommasina.perrone@policlinico.ba.it](mailto:tommasina.perrone@policlinico.ba.it); [pellegrino.musto@blu.it](mailto:pellegrino.musto@blu.it)
3. Department of Precision and Regenerative Medicine and Ionian Area, University of Bari, Bari Italy
4. Department of Medicine and Surgery, LUM University, Casamassima-Bari, Italy; E-Mails: [farella@lum.it](mailto:farella@lum.it); [martinotti@lum.it](mailto:martinotti@lum.it); [gaudio@lum.it](mailto:gaudio@lum.it); [fragaudio@alice.it](mailto:fragaudio@alice.it)

\* **Correspondence:** Francesco Gaudio; E-Mails: [gaudio@lum.it](mailto:gaudio@lum.it); [fragaudio@alice.it](mailto:fragaudio@alice.it)

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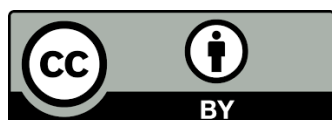
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**Abstract**

Primary mediastinal large B-cell lymphoma (PMLBCL) is an aggressive B-cell malignancy that may exhibit resistance to standard chemoimmunotherapy. Novel immunotherapeutic strategies, including checkpoint inhibitors and chimeric antigen receptor T-cell (CAR-T) therapy, are being explored in cases that are refractory to treatment. We report the case of a 29-year-old patient with refractory PMLBCL who was treated with pembrolizumab as a bridging therapy followed by axicabtagene ciloleucel (CAR-T) after failing R-CHOP and R-DHAP regimens. Initial disease progression following pembrolizumab was consistent with an immune-related flare phenomenon. Subsequent CAR-T infusion led to a complete metabolic



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response. Immunologic monitoring revealed expansion of CD3<sup>+</sup> and CD56<sup>+</sup> lymphocyte subsets, suggesting immune activation. Notably, the patient maintained normal endocrine function and conceived spontaneously three years post-treatment, delivering a healthy infant at term. This case highlights the potential synergy between checkpoint inhibition and CAR-T cell therapy in refractory PMLBCL, providing rare evidence of preserved fertility following intensive immunotherapy. Early recognition of immune flare and multidisciplinary management are critical in optimizing outcomes for young patients receiving novel immunotherapeutic approaches.

### **Keywords**

Primary mediastinal large B-cell lymphoma (PMLBCL); pembrolizumab; CAR-T therapy immunotherapy; refractory lymphoma; chimeric antigen receptor T-cell (CAR-T); B-cell malignancies; targeted therapy; cancer immunotherapy

## **1. Introduction**

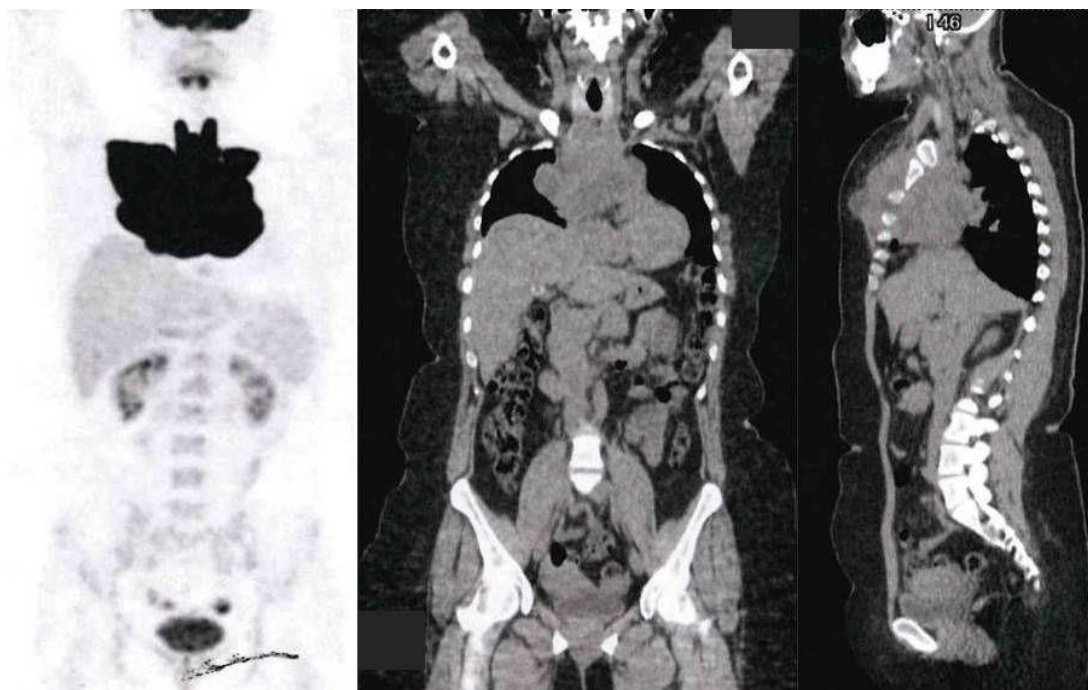
Primary Mediastinal Large B-Cell Lymphoma (PMLBCL) is a rare and aggressive subtype of non-Hodgkin lymphoma (NHL) that primarily affects young adults, typically presenting with a large mediastinal mass [1]. PMLBCL is known for its distinct clinical characteristics and relatively favorable prognosis [2]. However, some patients with PMLBCL develop refractory or relapsed disease, which presents significant therapeutic challenges [3-6]. In particular, the treatment of relapsed or refractory PMLBCL is difficult due to the aggressive nature of the disease and the lack of long-term responses to conventional therapies.

Recent therapeutic advancements, including the introduction of immune checkpoint inhibitors and chimeric antigen receptor T-cell (CAR-T) therapies, have offered new hope for patients with refractory or relapsed lymphoma. Pembrolizumab, an anti-PD-1 immune checkpoint inhibitor, has shown promise in various lymphoma subtypes [7] by enhancing immune system activity against tumor cells [8]. Similarly, CAR-T therapy has revolutionized the treatment of B-cell malignancies, including PMLBCL [9]. Despite these innovations, the management of aggressive lymphoma cases remains complex, particularly when initial responses are suboptimal or followed by disease progression.

## **2. Case Presentation**

### **2.1 Clinical Course**

A 29-year-old patient was diagnosed in October 2019 with stage IA PMBCL, presenting with a bulky anterior mediastinal mass (Figure 1) measuring 13 cm (transverse) by 11 cm (longitudinal), an International Prognostic Index (IPI) of 1, and elevated lactate dehydrogenase (LDH) levels.



**Figure 1** CT scan showing a large mediastinal mass measuring 13 cm in transverse diameter and 11 cm in longitudinal diameter in a 29-year-old patient diagnosed with stage Ia PMLBCL.

## **2.2 Immunophenotypic and Molecular Profile**

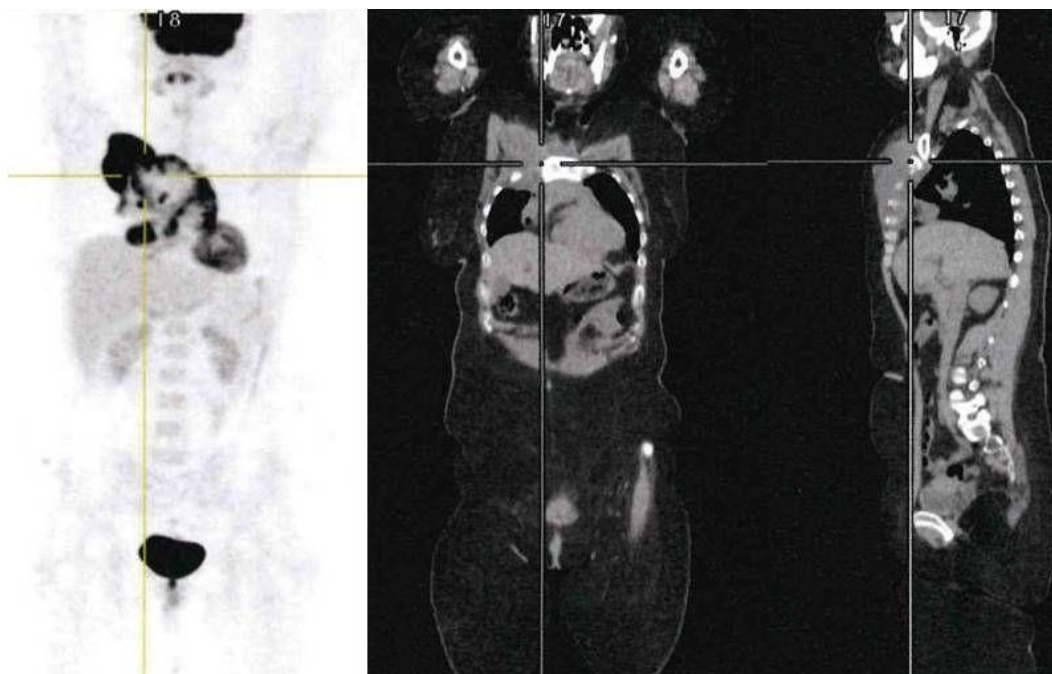
Histopathological examination revealed a diffuse proliferation of large atypical B cells with a high nuclear-cytoplasmic ratio and prominent nucleoli. Immunohistochemistry demonstrated expression of CD20, CD19, and CD79a, consistent with B-cell lineage. Neoplastic cells were also partially positive for CD30 (~30%), and positive for MUM1, while negative for CD10 and BCL6. PD-L1 expression exceeded 70% of tumor cells. The Ki-67 proliferation index was approximately 80%. EBER was negative. FISH analysis did not show rearrangements in MYC, BCL2, or BCL6.

## **2.3 Initial and Salvage Therapy**

The patient underwent six cycles of R-CHOP, achieving a partial remission with residual disease measuring 6 × 5 cm. Due to suboptimal response, two cycles of R-DHAP were administered; however, imaging revealed disease progression (8 × 7.7 cm).

## **2.4 Bridging and Advanced Therapy**

Given the refractory nature of the disease, bridging immunotherapy with pembrolizumab was initiated (four doses). Imaging showed an apparent increase in tumor size (13 × 15 cm), with central necrosis on PET/CT, consistent with a possible immune-related flare reaction (Figure 2).



**Figure 2** PET scan showing the mediastinal mass with increased size, measuring 13 cm in transverse diameter and 15 cm in longitudinal diameter, with a necrotic central area despite the increase in size.

Radiotherapy was delivered to the mediastinum using intensity-modulated radiotherapy (IMRT), at a total dose of 36 Gy in 18 fractions. Organs at risk were spared through conformal planning. Radiotherapy was performed in combination with ongoing pembrolizumab.

## **2.5 Fertility Evaluation**

A fertility preservation consultation was conducted before lymphodepletion. Hormonal evaluation showed normal values (FSH 4.8 mIU/mL, LH 5.2 mIU/mL, AMH 3.1 ng/mL, estradiol 45 pg/mL, testosterone 5.1 ng/mL). Follow-up assessments at 6 and 12 months confirmed preservation of gonadal function without evidence of premature ovarian insufficiency or hypogonadism.

## **2.6 Imaging Findings**

Baseline <sup>18</sup>F-FDG PET/CT demonstrated a hypermetabolic mediastinal mass (11.2 × 7.6 cm, SUVmax 18.6). Post-R-CHOP, the lesion reduced to 7.3 × 4.8 cm (SUVmax 9.1). Subsequent PET/CT at day +90 post-CAR-T showed a further decrease to 4.2 × 2.5 cm (SUVmax 3.7), and by day +180, the mass measured 2.1 × 1.1 cm with SUVmax 1.4, indicating complete metabolic response. Imaging was interpreted by two radiologists using standard software (MIM Software Inc.), with inter-observer variability estimated at ±3 mm.

## **2.7 CAR-T Cell Manufacturing and Infusion**

Leukapheresis was performed using the Spectra Optia system, yielding  $1.2 \times 10^9$  CD3<sup>+</sup> cells. The patient received axicabtagene ciloleucel (Yescarta®), with a dose of  $2 \times 10^6$  CAR<sup>+</sup> T cells/kg. Final

product viability exceeded 90%, with 47% transduction efficiency and 98% CD3<sup>+</sup> purity. All sterility tests were negative. Lymphodepletion was achieved with fludarabine (30 mg/m<sup>2</sup>) and cyclophosphamide (500 mg/m<sup>2</sup>).

CAR-T infusion occurred in January 2021. The patient experienced grade 1 cytokine release syndrome (CRS) and grade 1 immune effector cell-associated neurotoxicity syndrome (ICANS), both managed with supportive care.

## **2.8 Immunologic Response**

Peripheral blood immunophenotyping showed an increase in CD3<sup>+</sup> T cells from 650 to 1280 cells/ $\mu$ L (day +21), with a CD8<sup>+</sup> predominance. CD56<sup>+</sup> NK cells rose from 110 to 340 cells/ $\mu$ L. These immune changes were temporally associated with the flare phenomenon and preceded the radiological response.

## **2.9 Fertility Outcome**

Three years after CAR-T therapy, in January 2024, the patient conceived spontaneously. Pregnancy proceeded without complications and culminated in a full-term vaginal delivery of a healthy infant.

## **2.10 Ethical Statement**

This study was conducted according to the Declaration of Helsinki. Ethical review and approval were waived for this study, as the study data were collected during routine clinical activity and fully anonymized, thereby exempting formal institutional review board approval.

The patient enrolled in this study was informed about the procedures and signed a consent form, allowing for data collection and analysis for research purposes.

## **3. Discussion**

This case highlights the challenges in managing refractory primary mediastinal large B-cell lymphoma (PMLBCL) and underscores the potential role of novel immunotherapeutic approaches when conventional therapies fail [10, 11]. The integration of immune checkpoint inhibitors, such as pembrolizumab, and CAR-T cell therapy has substantially expanded treatment options for relapsed and refractory lymphomas, including PMLBCL [10, 11].

In this case, pembrolizumab was administered as bridging therapy after failure of both first- and second-line chemotherapy. Although early imaging suggested disease progression, subsequent findings and clinical stability pointed to an immune-related flare response. This phenomenon, characterized by transient tumor enlargement due to immune cell infiltration and cytokine release, is increasingly recognized in patients treated with PD-1 inhibitors. Mechanistically, immune flare is associated with the infiltration of activated T cells into the tumor microenvironment, accompanied by the release of cytokines such as IFN- $\gamma$  and TNF- $\alpha$ , increased vascular permeability, and peritumoral edema. These features can mimic disease progression on imaging and may lead to premature treatment discontinuation [12, 13].

Flare reactions have been documented in patients receiving anti-PD-1 therapies, particularly in Hodgkin lymphoma and certain solid tumors, and have also been reported following CAR-T cell

infusion, where immune reconstitution and transient inflammation contribute to similar radiologic findings. Differentiating actual progression from pseudoprogression requires a multimodal approach, incorporating clinical evaluation, PET/CT-based metabolic criteria (e.g., SUVmax, Deauville score), and longitudinal imaging. In uncertain cases, biopsy may be warranted to confirm disease status [12, 13].

The observed increase in CD3<sup>+</sup> and CD56<sup>+</sup> immune cell subsets, particularly CD8<sup>+</sup> T cells, following CAR-T infusion and during PD-1 blockade, reflects immune activation and supports the hypothesis of synergistic immunologic reprogramming. Anti-PD-1 agents reinvigorate exhausted T cells and modulate the tumor microenvironment by enhancing antigen presentation and reducing immunosuppressive elements, thereby potentially priming the host immune system for more effective CAR-T activity. CAR-T cells subsequently deliver a targeted cytotoxic response and may trigger epitope spreading, broadening anti-lymphoma immunity [14, 15]. However, the lack of cytokine analysis, immune repertoire profiling, or next-generation sequencing limits deeper mechanistic interpretation.

An additional notable aspect of this case is the preservation of fertility despite exposure to multi-modal therapy, including cytotoxic chemotherapy, immune checkpoint blockade, radiotherapy, and CAR-T cell therapy. Hormonal monitoring demonstrated preserved gonadal function, and the patient achieved a spontaneous, full-term pregnancy three years after treatment. This challenges the traditional assumption that intensive oncologic treatments inevitably compromise fertility, particularly in the context of novel immunotherapies with potentially lower gonadotoxic profiles.

While the reproductive impact of alkylating agents and radiotherapy is well established [16-18], the effects of checkpoint inhibitors and CAR-T therapy on fertility remain poorly defined. Preclinical data suggest that systemic immune activation can transiently impair gametogenesis, though reversibility is generally observed [15, 19]. Clinical data are limited but include reports of recovered endocrine function and rare cases of successful pregnancy post-treatment. Given these uncertainties, fertility preservation strategies should be discussed with all young patients eligible for immunotherapy.

Long-term fertility data in patients treated with immunotherapy and CAR T-cell therapy for lymphoma are minimal, and there are no established guidelines for fertility preservation [20, 21]. Spontaneous pregnancies following CAR T-cell therapy are rarely reported in the literature. A survey conducted across 66 centers using CAR T-cell therapy found that seven patients became pregnant, resulting in a total of five live births. Only two of these live births occurred utilizing the patient's oocytes [22]. Therefore, further research is needed on long-term fertility outcomes in both men and women who receive CAR T-cell therapy. Our case provides a significant example of the potential for spontaneous pregnancies in women with PMLBCL treated with chemotherapy and immunotherapy but cured with CAR T-cell therapy. Furthermore, new guidelines recommend CAR T-cell therapy for patients who are not candidates for high-dose chemotherapy with stem cell rescue, increasing the number of patients who may present with cases similar to ours.

In conclusion, this case exemplifies the therapeutic and biological complexity of treating refractory primary mediastinal large B-cell lymphoma with sequential immunotherapy [23, 24]. The combination of PD-1 blockade and CD19-directed CAR-T cell therapy not only resulted in durable remission but also demonstrated the potential immunologic synergy between these modalities. Importantly, the occurrence of an immune-related flare followed by sustained

response underscores the need for careful interpretation of early imaging during immunotherapy and highlights the value of clinical context in guiding treatment decisions [12-14].

Equally significant is the preserved reproductive potential observed in this case. Despite exposure to multi-line chemotherapy, radiotherapy, checkpoint inhibition, and CAR-T therapy, the patient maintained normal endocrine function and achieved a successful spontaneous pregnancy. This outcome challenges conventional assumptions about gonadotoxicity in intensive cancer therapies and suggests that immunotherapy-based regimens may offer a more favorable reproductive profile compared to traditional approaches [25-28].

This case reinforces the importance of a multidisciplinary strategy that incorporates clinical immunology, reproductive counseling, and longitudinal monitoring. Future studies should aim to validate the immunologic markers of flare and durable response, as well as establish prospective registries for fertility outcomes in patients undergoing immunotherapy. Collectively, such efforts will help refine treatment algorithms and support survivorship planning for young patients with hematologic malignancies.

### Author Contributions

FEL, SM and FG conceived and designed the study. TP, PM, IF acquired the data. FEL and FG drafted the manuscript. All authors revised the manuscript. All authors read and approved the final manuscript.

### Competing Interests

All authors declare no conflict of interest.

### Data Availability Statement

The relevant data have been included in the manuscript. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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