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ANALYSIS OF EFFICACY AND SAFETY OF CAR T-CELL THERAPY IN RELAPSED/REFRACTORY LYMPHOMAS: CURRENT INDICATIONS AND FUTURE PERSPECTIVES

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INTRODUCTION

Large B-cell lymphomas (LBCL) represent the most common forms of lymphoproliferative diseases and they encompass a wide range of aggressive neoplasms, characterized by a rapidly progressive course and associated with different prognostic features [1]. Although the significant progress made during the past decades has led the majority of patients to be cured with frontline immunochemotherapy, approximately 30-40% of them do not respond properly or eventually relapse, representing an extremely high-risk population who can hardly be saved with conventional treatment strategies [2,3].

In the recent years, the advent of chimeric antigen receptor (CAR) T-cells has substantially enriched the therapeutic armamentarium of these individuals, who long represented a major unmet medical need [4,5]. CAR T-cells are autologous cellular products derived from the patients' T-lymphocytes, which are engineered with the ability of recognizing a specific target on neoplastic cells and consequently directing their cytotoxic activity against the tumor [6].

The first two second-generation CAR T-cell products to be introduced in the clinical practice for LBCL patients were axicabtagene ciloleucel (axi-cel) and tisagenlecleucel (tisa-cel), both targeting the cluster of differentiation (CD)-19 antigen on the surface of malignant cells. Their indications expanded to different subtypes of LBCL over time; primary mediastinal B-cell lymphoma (PMBCL), however, is only included in the axi-cel indications. The trials that led to the approval of axi-cel and tisa-cel for LBCL reported an overall response rate (ORR) of 50-80% with a complete response rate (CRR) of 40-58% and a 1-year overall survival (OS) of approximately 50% in a population of heavily pre-treated patients, making CAR T-cell therapy the most promising strategy in this difficult setting [7,8].

Since CAR T-cells approval by regulatory agencies worldwide, it has become clear that the collection of real-life data was crucial to understand whether the exceptional results obtained in the pivotal trials were reproducible outside of the studies' stringent inclusion criteria, and also to better define the management of adverse events (AEs) [9,10].

I herein report the data collected at the “L. e A. Seràgnoli” Haematology Institute in Bologna since August 2019, when we first began employing this treatment strategy in LBCL patients. Ours was the first real-life Italian experience to be presented and published [11], and the constant expansion of our case series allows for an ever-increasing understanding of the feasibility of this innovative therapy outside of clinical trials.

1. LARGE B-CELL LYMPHOMAS

The 5th World Health Organization (WHO) classification of haematological malignancies (WHO-HAEM5) recognizes 18 diverse subtypes of LBCL, which share some histopathological and clinical features and are generally characterized by an aggressive behaviour, although the genetic profile and prognosis of the different entities varies significantly [1]. I will herein concentrate on the description of the LBCL subtypes that were included in the indications of CAR T-cell products according to the Italian Medicines Agency (*Agenzia Italiana del Farmaco*, AIFA) reimbursement list since the initial approval of axi-cel and tisa-cel, and that we therefore treated at our Institute since August 2019: diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS) and PMBCL.

1.1 DIFFUSE LARGE B-CELL LYMPHOMA

1.1.1 Epidemiology, pathological features and molecular classification

DLBCL, NOS is the most common subtype of LBCL and the most common form of non-Hodgkin lymphoma (NHL), representing approximately 30% of all cases [12]. Median age at diagnosis is around 65 years, with about 1/3 of patients being older than 75 years. While the majority of patients present with a recent history of rapidly progressive lymphadenopathies in the absence of a prior history of haematologic malignancies, in a smaller proportion of individuals the disease derives from the aggressive transformation of an indolent form of NHL, such as follicular lymphoma (FL) or marginal zone lymphoma (MZL) [12].

DLBCL, NOS itself encompasses different disease subtypes, that can be distinguished on the basis of morphological, immunohistochemical, genetic and molecular analysis [13].

DLBCL forms are generally composed of medium to large-sized cells with round to ovoid nuclei and vesicular chromatin, although cases with intermediate-sized cells and blastoid appearance may occur [1]. Considerable efforts are constantly

applied to perfecting the distinction between these subtypes, since it has become clear that an accurate classification is crucial for prognostic stratification and optimization of treatment strategies [12–14].

Gene expression profiling (GEP) studies have outlined two major molecular subgroups of DLBCL, distinguished on the basis of the stage of lymphoid differentiation of their cell of origin (COO): the germinal centre B-cell – like (GCB) subtype, with a gene signature typical of germinal centre B cells, and the activated B-cell – like (ABC) subtype, characterized by constitutive activation of B-cell receptor (BCR) signaling and activation of the nuclear factor-κB (NF-κB) pathway. Approximately 10-15% of cases remain unclassifiable [1,12]. Since the GEP techniques are time-consuming and costly, however, the immunohistochemistry-based Hans algorithm is generally employed in clinical practice, as it demonstrated to be able to reliably differentiate between GCB or non-GCB (ABC and unclassifiable) cases with a concordance of around 80% to GEP [12,15]. ABC – like DLBCL tends to be associated with inferior outcomes compared to GCB forms, with progression-free survival (PFS) at 3 years of 40-50% and 75%, respectively [12,16].

A more thorough and accurate classification of DLBCL subtypes can be obtained with molecular approaches such as next generation sequencing (NGS), which led to the development of systems that subdivide DLBCL into separate groups according to the prevalent signaling pathway involved in the pathogenesis [17–20].

In addition, fluorescence *in situ* hybridization (FISH) allows for the detection of recurrent genetic aberrations that yield a substantial prognostic significance. The oncogene *MYC*, for instance, is rearranged in about 12% of cases; in 4-8% of cases, this coexists with rearrangements in *B-cell lymphoma* (*BCL*)*2* and/or *BCL6* genes [12,21]. The most frequent translocation partner is an immunoglobulin (Ig) gene, which associates with adverse outcome [12]. Cases with concomitant mutations of *MYC* and either *BCL2* are not classified as DLBCL, NOS but as DLBCL/high-grade B-cell lymphomas (HGBL) with *MYC* and *BCL2* rearrangements, commonly referred to as double-hit lymphomas (DHL) [1,21]. Forms with concurrent rearrangement of *MYC* and *BCL6* and/or *BCL2* are enlisted among HGBL, NOS;

the presence of all three aberrations (*MYC*, *BCL2* and *BCL6*) defines the triple-hit lymphoma (THL) subtype [1]. While the majority of DHL/THL also overexpress MYC and BCL2/BCL6 at a protein level, the presence of protein overexpression (which can also be secondary to gene amplification or post-translational processes) does not necessarily correlate with the presence of genetic aberrations.[1,21] The overexpression of the MYC and of the BCL2 proteins (threshold set at ≥ 40% for MYC and > 50% for BCL2), without the underlying genetic aberrations, occurs in 45% and 65% of cases, respectively. DLBCL with concomitant expression of both products (around 30% of cases) is termed dual expressor lymphoma (DEL), which is not considered a separate entity, although it is known to have a more unfavourable prognosis compared to DLBCL, NOS. Curiously, the 20% of DHL cases that do not show protein overexpression seem to have improved outcome [21].

1.1.2 Clinical presentation, staging and prognostic factors

Commonly, DLBCL, NOS presents with the quick, progressive enlargement of lymphadenopathies that can be superficial, thereby being promptly noticed by the patient, or localized within the abdomen or chest, thus becoming manifest only when symptoms appear. Apart from lymph nodes, any other tissue and organ can be involved with the disease; in fact, up to 40% of cases are characterized by extranodal localizations, whereas only in about 20% of cases is DLBCL localized to a single anatomical site (nodal or extranodal) [22]. Approximately one third of patients complain of systemic symptoms, the so-called B-symptoms, which are represented by fever (usually in the evening or night), night sweats or weight loss of >10% of body weight during the 6 months preceding diagnosis. Local symptoms can also be present depending on the specific disease localizations [22]. DLBCL can also localize to the central nervous system (CNS); this particular presentation is now included in a specific WHO-HAEM5 category, termed LBCL of immune-privileged sites. CNS DLBCL represents < 1% on all NHL and around 2-3% of all brain neoplasms. These forms, globally associated with poor prognosis, require treatment with specific drugs that, at a certain high dose, are able to cross the blood-brain barrier (BBB) [22].

Staging of these disease require both laboratory and imaging procedures. Alterations of the patient's complete blood count (CBC) can reveal a bone marrow localization; lactic dehydrogenase (LDH) and uric acid are markers of the tumor burden; assessment of hepatic and renal function, as well as complete serology for hepatits B and C viruses (HBV, HCV), human immunodeficiency virus (HIV), *Treponema pallidum* and a QuantiFERON blood test are fundamental before starting a systemic treatment [12,22]. A computed tomography (CT)-scan and a positron emission tomography (PET)-scan with ^{18}Fluorodeoxyglucose (FDG) are nowadays considered standard staging procedures and allow for the detection of basically every disease localizations. A bone marrow trephine biopsy should also be performed; additional tests can be necessary in presence of particular disease presentations, such as CNS or testicular involvement [22]

DLBCL, NOS should be staged in accordance with the standard Ann Arbor criteria and the Lugano Classification, which reflect the number and localization of involved anatomical sites, the presence of extranodal disease and the existence of B-symptoms. Stage I refers to the involvement of a single nodal region or extranodal organ (in this case, I_E); stage II indicates the involvement of ≥ 2 nodal regions on the same side of the diaphragm (with possible extension to a contiguous extranodal tissue, II_E); stage III implies the involvement of nodal regions on both sides of the diaphragm, with possible splenic (III_S) or contiguous extranodal localization (III_E); stage IV means that the disease shows disseminated extranodal localizations, with or without lymph node involvement [23–26].

Despite the well-known importance of genetic and molecular characteristics in determining the patients' outcome, these biological features are still excluded from the commonly used prognostic indexes. Three scores are currently available: the International Prognostic Index (IPI), introduced 30 years ago and identifying 4 risk categories; the revised-IPI, developed in the era of monoclonal anti-CD20 antibody rituximab (R) and distinguishing 3 risk groups; the National Comprehensive Cancer Network IPI (NCCN-IPI), the most recently introduced system, recognizing 4 risk categories [27–29]. All 3 scores require the measurement of a few clinical and laboratory features that are easily obtained through the

common diagnostic procedures (namely age, LDH, extranodal involvement, Ann Arbor stage, and performance status); the most relevant differences depend on the calculation methods used in the scores [30]. A recent comparison of the three systems has shown the superiority of the NCCN-IPI, although none of them proved able to identify a very poor-risk group with long-term overall survival (OS) < 50% [30]. The integration of molecular and genetic features into a clinical and biological prognostic model could help overcome these limitations [12,30].

In addition, a specific model has been developed to assess the risk of CNS relapse or progression in patients with DLBCL treated with standard immunochemotherapy. The 5-risk factors CNS-IPI index stratifies patients in three risk groups: patients in the low-risk group (0-1 points) have a 2-year probability of 0.6% of developing CNS progression, while patients in the intermediate-risk group (2-3 points) and in the high-risk group (4-6 points) have a probability of 3.4% and 10.2%, respectively [31].

1.1.3 Frontline treatment

For young, fit patients, the frontline treatment of DLBCL, NOS relies on the employment of an anthracycline-containing chemotherapy regimen, namely cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP regimen), combined with rituximab (R-CHOP) and administered every 3 weeks for a total of 6 cycles [32–35]. Considering the know cardiotoxic effects of anthracyclines, elderly patients or individuals with cardiac comorbidities usually receive a liposomal formulation of doxorubicin, which is associated with a reduced risk of short- and long-term cardiac complications [36]. No clinical trial has demonstrated an advantage in administering 8 instead of 6 cycles of R-CHOP [34]; similarly, neither dose-intensive regimens nor attempts at therapy intensification with upfront autologous stem cell transplantation (ASCT) have yielded substantial survival advantage, especially considering the significantly increased toxicity [37,38].

Radiotherapy (RT) can benefit patients with residual disease detected on the post-treatment FDG-PET scan, while it can be safely omitted in those achieving a complete metabolic response (CMR) [12,39]. Moreover, individuals presenting with

non-bulky (largest mass measuring < 7.5 cm), limited stage (Ann Arbor I-II) disease, who account for about 30% of DLBCL patients and generally have low-risk clinical features and favourable prognosis, can benefit from a reduced number of immunochemotherapy cycles (generally 3) in exchange for the addition of involved-field (IF)-RT, granted they achieve CMR on the PET-scan performed after the third immunochemotherapy cycle [40,41].

In the last decades, the ever-improving knowledge of DLBCL biology has led to the design of various trials aiming at optimizing frontline treatment by adding novel agents to the R-CHOP backbone. Bortezomib, ibrutinib and lenalidomide could have a role in ABC-like DLBCL, where the BCR and NF-κB signaling pathways are particularly active, but randomized trials have failed to show a significant improvement in survival outcomes [42–45].

Among the novel agents, the anti-CD79b antibody-drug conjugate (ADC) polatuzumab vedotin (pola) has been demonstrated to grant a PFS benefit in newly diagnosed DLBCL patients when added to frontline conventional immunochemotherapy: the POLARIX trial reported a significant increase in 2-year PFS from 70.2%, obtained in the R-CHOP arm, to 76.7% ($p = 0.02$) in the experimental arm with pola-R-CHP (vincristine omitted because of overlapping neurotoxicity) [46]. The reported OS, however, was similar between the two cohorts probably due to short follow-up of 28 months. The advantage was more evident in patients older than 60 years, with IPI score between 3 and 5, non-bulky disease and non-GCB subtype of DLBCL [46].

1.1.4 Relapsed and refractory disease

Despite the remarkable results obtained with frontline R-CHOP, approximately 30-40% of patients with DLBCL, NOS are not cured by this treatment strategy: 10-15% of them have primary refractory disease (defined as an incomplete response or a relapse within 6 months from first-line therapy) while 20-25% of them relapse after an initial response, usually within the first 2 years, and their outcome is mostly unfavourable with median OS of approximately 6 months [3].

Approximately 50% of relapsed or refractory (R/R) patients can be considered transplant-eligible because of young age and overall fitness, and they can be effectively cured with HDC and ASCT provided the disease is sensitive to the chemotherapy salvage regimen, which is the case for around half of them [12]. A platinum-based, rituximab-associated schedule is generally considered gold-standard in this setting, with no significant differences in efficacy between the various combinations [2,47]. Overall, the cure rate after ASCT is approximately 25-35% [2,12,47].

A large amount of patients, however, are not eligible for transplantation due to age or comorbidities, poor performance status or lack of response to salvage HDC [12]. In the last decades, numerous chemo-free therapeutic options have been explored, more or less successfully, and some of them have been included into the clinical practice.

Lenalidomide, an immunomodulatory agent with multifaceted activity in hematologic malignancies, has demonstrated modest single-agent activity in aggressive lymphomas in a phase 2 study by Wiernik et al. that included a cohort of 26 patients with DLBCL, NOS; among these, ORR was 19% with 1 patient achieving CR. The main AEs were represented by haematological toxicity and infections [48]. Another phase 2 trial reported similar outcomes with ORR 28% and CRR 7% among DLBCL, NOS patients; median PFS and median DOR were only 2.7 months and 4.6 months, respectively [49]. The synergy between lenalidomide and rituximab was initially observed in preclinical studies [50] and subsequently tested in clinical trials, which obtained better results compared to lenalidomide monotherapy with ORR of 28-35% and CRR of 22-30%; moreover, patients who manage to achieve a CR have a good chance of maintaining it indefinitely [51,52].

The anti-CD19 monoclonal antibody tafasitamab has shown single-agent activity in the phase 2 study by Jurczak and coworkers, with ORR of 26% and CRR of 6% among the cohort of patients with DLBCL, NOS. Responses were rather durable, especially for patients obtaining a CR, with median duration on response (DOR) of 20.1 months; with a median follow-up time of 21 months, median PFS was 2.7 months [53]. More importantly, tafasitamab demonstrated a synergistic effect when

combined with lenalidomide in the phase 2 study L-MIND, which reported an ORR of 60% and CRR of 43% along with a good safety profile. Median PFS was 12.1 months, while median OS was not reached at a median follow-up of 19.6 months [54]. The durability of responses was confirmed at the updated analysis published in 2021 after a follow-up longer than 35 months: median DOR was 43.9 months and median OS was 33.5 months; median PFS was 11.6 months, and significantly longer in patients receiving the tafasitamab-lenalidomide combination as second-line therapy instead of later lines (23.5 months vs 7.6 months) [55]. Overall, the regimen has a good safety profile and favourable efficacy, although it is not clear whether it can actually benefit patients with primary refractory disease or high-risk groups such as DHL and THL, since the proportions of these subgroups in the L-MIND study were very small [54]. Therefore, the current recommendation is to use the tafasitamab-lenalidomide combination as second-line strategy in transplant-ineligible DLBCL, NOS patients who have relapsed after >12 months after frontline therapy [56].

Loncastuximab tesirine is another ADC recently introduced into clinical practice. The drug binds to the CD19 surface antigen, thus selectively delivering a molecule of cytotoxic agent (represented by a molecule of pyrrolobenzodiazepine) into the tumour cell [12]. The phase 1, dose-escalation and dose-expansion study of loncastuximab tesirine reported an ORR of 42.3% among the DLBCL, NOS subgroup, including 23.4% CR. In DLBCL, NOS patients, median PFS was 2.8 months and median OS was 7.5 months; median DOR was 4.5 months, but median duration of CR (DOCR) was not reached for individuals who received doses above 120 μg/kg. Treatment-emergent AEs (TEAEs) included oedema and effusions, liver enzyme abnormalities, rash and photosensitivity [57,58]. These encouraging results prompted the design of the LOTIS-2 phase 2 study on DLBCL, NOS patients at the dose of 150 μg/kg for the first 2 doses infused at a distance of 21 days, followed by a dose of 75 μg/kg administered intravenously on day 1 of every 21-day cycles. ORR was 48.3% and CRR 24.1% in a population of heavily pretreated patients who had received a median of 3 previous lines of therapies [59]. The updated analysis published this year showed that CRs tend to be durable, with

44% and 31% of CR patients remaining event-free for ≥ 1 year and ≥ 2 years, respectively. Median DOR and median PFS in the whole population were 13.4 months and 4.9 months, respectively, while they were not reached for CR patients; likewise, median OS was 9.5 months for the all-treated population and was not reached in patients with CR [60]. The study included 14 patients (9.7%) previously treated with CAR T-cells, who obtained an objective response in 42.9% of cases, while 16 patients received CAR T-cell therapy after loncastuximab tesirine. These preliminary data suggest that the two CD19-directed strategies are not necessarily mutually exclusive, which is consistent with previously reported results that CD19 loss is unusual after loncastuximab exposure [59]. A subgroup analysis was conducted on the 15 patients with HGBL enrolled in the LOTIS-2 trial: 5 patients had an objective response (ORR 33.3%), which was a CR in all 5 cases. All responses lasted longer than 12 months, with a median DOR not reached at the time of data cutoff, while median PFS and OS were 3.7 and 9.2 months, respectively (median follow-up of 5.8 months)[61]. The ongoing phase 2 LOTIS-3 trial (NCT03684694) is currently exploring the combination of loncastuximab tesirine with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib in patients with R/R DLBCL or mantle cell lymphoma (MCL), and results are promising with 58.6% ORR and 31% CRR among DLBCL patients [62].

Another important category of novel agents is represented by bispecific monoclonal antibodies (BsAbs) targeting CD20 on lymphomatous cells and CD3 on normal T lymphocytes. These drugs induce T-cell activation by engaging malignant B-cells and thus lead to the death of neoplastic lymphocytes via cell-mediated cytotoxicity [12]. This peculiar mechanism of action, common to other immunotherapies exploiting the same principle (such as CAR T-cells), commands a widespread immune system activation with release of cytokines by activated lymphocytes, myeloid cells and also non-immune cells, such as endothelial cells. This phenomenon, initially described as a "cytokine storm", is responsible of both the effectiveness of BsAbs and their particular toxicity profile, which is characterized by two main types of AEs: cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity (ICANS) [63,64]. In order to mitigate

the incidence and severity of these events, an initial step-up dosing (SUD) schedule has been studied for BsAbs in dose-finding trials [63,65]. The fully humanized IgG1 BsAb mosunetuzumab was tested in a first-in-human phase 1/1b trial enrolling 129 patients with aggressive NHL, including 82 patients with R/R DLBCL, NOS. Among patients with aggressive NHL, ORR was 35% with 19.4% CRR; median DOCR was 22.8 months [66]. In a phase 1/2 trial on 88 patients with heavily pretreated R/R DLBCL, NOS, mosunetuzumab provided an ORR of 42% and CRR of 24%. With a rather short median follow-up of 10.1 months, median PFS and OS were 3.2 months and 11.5 months, respectively, and around 70% of complete responders maintained the CR at 12 months after the first response [67]. Glofitamab has a peculiar 2:1 configuration which confers bivalency for CD20 and monovalency for CD3. The drug displayed impressive single-agent activity in a phase 1 dose-escalation and expansion study that enrolled 171 heavily pretreated patients, approximately 70% of whom with a diagnosis of DLBCL, NOS. The ORR was 53.8% (CRR, 36.8%) among all doses and 65.7% (with 57% CRR) in those dosed at the recommended phase 2 dose (RP2D); 53/63 (84.1%) patients with a CR are disease-free after a maximum follow-up of 27.4 months [68]. In addition to the SUD, patients receive a single infusion of obinutuzumab 1000 mg on the first day of cycle 1 (7 days prior to the start of glofitamab) in order to produce a clearance of B-cells and thus reduce the risk of high-grade CRS [68]. A subsequent phase 2 study on 154 DLBCL, NOS patients showed an ORR of 52% with 39% CRR. At a median follow-up of 12.6 months, median PFS was 4.9 months; 64% of patients had an ongoing objective response and CR was ongoing in 78% of patients; the median DOCR was not reached [69].

Epcoritamab is a full-length IgG1 bispecific antibody comprised of a humanised murine-derived anti-human CD3 moiety and a human anti-CD20 component. Differently from the previously described BsAbs, epcoritamab is administered subcutaneously and does not have a fixed-duration schedule: treatment is continued until disease progression or unacceptable toxicity [70]. In the phase 1/2 study, a cohort of 46 DLBCL, NOS patients received epcoritamab and obtained an ORR of 68%, with 45% achieving a CR; specifically, at the RP2D of 48 mg the

ORR was 88% with 38% of patients achieving a CR [70]. Results from the LBCL expansion cohort (157 patients) were consistent, with 63% ORR and 39% CRR; median DOR was 12 months, while median DOCR was not reached. Median PFS was 4.4 months, and not reached among complete responders; median OS was not reached, as well [71]. Overall, BsAbs display a lower incidence of serious CRS and ICANS events compared to CAR T-cells [66,69,70].

1.2 PRIMARY MEDIASTINAL B-CELL LYMPHOMA

1.2.1 Epidemiology, pathological features and molecular characteristics

PMBCL is a rare subtype of LBCL, accounting for approximately 5% of all NHL and clearly distinguished form other LBCL forms on the basis of clinical and biological features. It is typically diagnosed in young individuals in their third or fourth decade and it affects women twice as much as men (in the white population, at least) [1,72].

From a histological point of view, it is characterized by the diffuse growth of neoplastic cells (likely derived from thymic medullary B cells) in a context of bands of sclerotic tissue determining compartmentalization [73,74]. The CD30 antigen is expressed in the majority of cases (around 80%), although with a weaker and more heterogeneous staining compared to what is generally found in Hodgkin's disease (HD) [72,73].

PMBCL cells owe their survival and proliferation advantage to certain recurrent genetic aberrations. The chief biological feature, which PMBCL shares with classical HD, is the amplification of the chromosome band 9p24.1, recognised in 50–70% of cases and leading to an enhanced expression of Janus kinase 2 (JAK2) with consequent activation of the JAK-signal transducer and activator of transcription (JAK/STAT) pathway. The constitutive activation of the NF-κB pathway is another key element of the PMBCL pathogenesis and it is sustained by additional genetic abnormalities, among which the amplification of the *REL* locus

on chromosome 2p [73,74]. The amplification of 9p24.1 also determines the presence of copy number alterations and rearrangements of the *programmed cell death ligands* (*PD-L*)*1* and *PD-L2* genes, resulting in the overexpression of PD-L1 and PD-L2 on lymphoma cells [74,75]. The JAK/STAT pathway itself contributes to upregulating the expression of these molecules that, by interacting with the PD-1 antigen on tumour-infiltrating T-lymphocytes, mediate T-cell anergy through the release of pro-survival signals. This results in the creation of an immunotolerant microenvironment, where neoplastic cell can elude the host's immune response [72,74].

1.2.2 Clinical presentation, staging and prognostic factors

Patients with PMBCL usually present with rapidly enlarging bulky anterior mediastinal masses, frequently infiltrating or compressing adjacent structures and quickly determining local symptoms, very often a superior vena cava syndrome. The disease rarely involves extrathoracic lymph nodes or organs at the time of diagnosis; distant localizations, including extranodal sites such as CNS structures, liver, ovaries, kidneys and adrenal glands are more commonly observed at relapse [72,73].

The initial work-up of PMBCL should include a CT- and an FDG-PET-scan, along with routine blood tests and possibly a bone marrow trephine biopsy. The recommended staging system is based on the standard Ann Arbor classification; due to the abrupt development of symptoms, the disease is generally diagnosed at an early stage (often stage II) [23–26].

The IPI models extensively used for DLBCL have limited utility in PMBCL, probably because of the age distribution of the disease and the usual early stage at diagnosis. A population-based study from the British Columbia found that elevated LDH to ≥ 2 the ULN, age over 40 and ECOG PS ≥ 2 correlate with reduced survival; however, a large series from the International Extranodal Lymphoma Study Group (IELSG) reported male sex, poor performance status and advanced-stage disease as significant unfavourable predictors [76].

1.2.3 Frontline treatment

The mainstay of PMBCL frontline management is represented by an anthracycline-containing chemotherapy regimen with addition of rituximab; however, there has been much debate over the years concerning which type of schedule should be selected and whether it should be accompanied by consolidative RT [72,76,77]. Since the eighties, several experiences (mainly retrospective) have been published showing that dose-dense third-generation (alternating) regimens, such as methotrexate/etoposide, doxorubicin, cyclophosphamide, vincristine, bleomycin and prednisone (M/VACOP-B) could yield better results than CHOP administered every 3 weeks (CHOP-21), both in terms of remission rates and survival [72,77]. In 2002, the IELSG published a retrospective analysis which clearly stated the superiority of third-generation regimens over the CHOP-21 schedule, with higher CRR (79% and 61% for MACOP-B/VACOP-B and CHOP, respectively) and significantly longer 10-year PFS (67% versus 35%). The same study also showed that RT could have an important role in response consolidation: among the 148 patients who achieved a partial response (PR), 124 (84%) underwent radiation therapy and 100 (81%) of them converted their response to a CR, as documented by gallium (^{67}Ga) scans [78]. The dose-adjusted etoposide, doxorubicin, cyclophosphamide, vincristine and prednisone (DA-EPOCH) regimen was also found to provide excellent results in PMBCL, either alone or in combination with rituximab [79,80]. The immunochemotherapy association, in particular, was able to prevent the need for RT in 96% patients with PMBCL in a phase 2 study by Dunleavy and coworkers and led to event-free survival (EFS) and OS rates of 93% and 97%, respectively [80]. On the contrary, the addition of rituximab to MACOP-B/VACOP-B chemotherapy (plus RT on PET-positive residues) was not found to improve results in PMBCL in terms of CR, relapse-free survival (RFS) and disease-free survival (DFS) rates [81,82].

The role of RT remains a controversial issue in the PMBCL management because, while it has the power to perfect responses in patients with residual FDG-avid mediastinal masses, it is also associated with significant long-term toxicity such as

second malignancies and accelerated coronary artery disease [82]. Great effort has gone into identifying the patients who actually benefit from the addition of RT. FDG-PET scan has emerged as a powerful tool in this setting: the Deauville five-point scale [83] can be used to stratify patients on the basis of metabolic response, which correlates with outcome, in order to adapt the treatment strategy [82,84]. The idea of a PET-guided consolidative RT has been anticipated by single-centre experiences [82,85] and recently validated by the largest prospective trial ever conducted on PMBCL patients: the IELSG-37 study, whose results were presented at the 2023 Meeting of the American Society of Clinical Oncology (ASCO), showed that RT can be safely omitted in individuals achieving CMR (defined as Deauville score 1-3 according to the Lugano classification), after immunochemotherapy [86].

1.2.4 Relapsed and refractory disease

Although frontline treatment of PMBCL yields a high cure rate, around 15-20% of patients are refractory to it or eventually relapse, usually within the first 18 months of follow-up [72]. Although the standard of care for the second-line therapy of these patients is still represented by an intensive chemotherapy regimen followed by HDC and ASCT, it is by now well-established that R/R individuals tend to be chemorefractory and seldom reach the CR status that is crucial for proceeding to ASCT consolidation [72,87].

Therefore, given the peculiar biology of PMBCL and its almost invariable albeit heterogeneous expression of CD30, novel agents have been explored in the setting of R/R disease. The anti-CD30, MMAE-coupled ADC brentuximab vedotin (BV) showed objective responses in CD30-positive R/R LBCL patients (including 6 patients with PMBCL) enrolled in a phase 2 study, with an ORR of 17% and half of the patients maintaining a disease stability. Interestingly, the responses did not correlate with the quantitative CD30 expression on tumor cells [88]. These results prompted the design of an open-label phase 2 study by the Italian Cooperative Study Group on Lymphomas (*Fondazione Italiana Linfomi*, FIL), which enrolled 15 patients with CD30-positive PMBCL who were R/R to induction treatment [89].

The ORR was 13.2%, with only 2 patients obtaining a PR, 1 patient having a stable disease (SD) and all 12 remaining patients progressing while on treatment. It is unclear whether the substantial inactivity of BV monotherapy in PMBCL could depend on the CD30 expression pattern in this subtype of disease [72].

On the other hand, considering the high dependence of the disease on the PD-1/PD-L1 or PD-L2 interaction, it comes as no surprise that the anti-PD-1 monoclonal antibodies nivolumab and pembrolizumab are able to provide very good responses in PMBCL patients. The multicohort phase 1b KEYNOTE-013 trial studied the employment of pembrolizumab in patients with R/R haematological malignancies, including 21 patients with PMBCL who displayed an ORR of 48% and CRR of 33%. Median DOR was not reached and 78% of patients had a response lasting over 12 months; median OS was reached at 31.4 months [90,91]. Following these initial results, the phase 2 KEYNOTE-170 trial was designed and enrolled 53 patients with R/R PMBCL patients, who received pembrolizumab at the fixed dose of 200 mg every 3 weeks for a maximum of 35 cycles or until disease progression, unacceptable toxicity, or patient withdrawal [91]. The ORR was 41.5%, with 11 patients (20.8%) achieving a CR; 76% of patients had a response duration longer than 12 months; median OS was 22.3 months (OS rate 45% at 4 years). Of the 18 patients who had a first response of PR, 7 subsequently improved to CR [92]. The toxicity profile of pembrolizumab was similar to what had already been reported in other settings and was overall manageable [90–92].

Studies on patients with R/R HD have led to discover a synergy between BV and nivolumab, not accompanied by an overlap of toxicity profiles: in addition to its direct cytotoxicity, BV seems to be able to activate the innate immune system and lead to response through the induction of immunogenic cell death [93]. The phase 1/2 CheckMate 436 study explored this combination in a population of 30 heavily pretreated patients with PMBCL and reached an ORR of 73% with 37% CRR. Median DOR, PFS and OS were not reached at a median follow-up of 11.1 months [94]. The 3-year update of the study, published earlier this year, reported a median PFS of 26 months after a follow-up of 39.6 months, while median OS was not reached. At 24 months, PFS and OS were 55.5% and 75.5%, respectively[95].

2. CAR T-CELL THERAPY

2.1. DEVELOPMENT AND STRUCTURE OF CAR T-CELLS

The last 20 years have led to a substantial revolution in the treatment approach to neoplastic diseases, especially in the haematological field. The constantly deepening knowledge of cancer's immune and molecular mechanisms has allowed for the development of innovative strategies that now side with, and sometimes substitute, the more traditional instruments represented by chemo- and radiotherapy. Since 1997, when rituximab received FDA approval for the treatment of FL and became the first monoclonal antibody to ever be employed as anti-neoplastic treatment [96], immunotherapy has been extensively studied and gradually added to the backbone of anticancer management.

Our immune system is not only devoted to responding to external threats and protecting us from infections, but it is also able to detect potential cancer-initiating cells and to destroy them before an actual malignant condition originates [97]. Tumor cells, however, have the ability to put in place various mechanisms of immune evasion: the loss of tumor-associated antigens (TAAs); the down-regulation of the major histocompatibility complex (MHC) molecules; the reduction of T-cell receptor (TCR) signaling; the production of immunosuppressive cytokines, such as transforming growth factor (TGF)-β or interleukin (IL)-10. This condition leads to the inactivation of tumor-specific T-cells and translates into a state of immune tolerance [97,98].

Hence came the idea of potentiating the immune system through the genetic manipulation of T-cells, in order to redirect them towards a specific TAA expressed on the neoplastic clone [97]. The first successful attempt at an adoptive cellular therapy (ACT) concerned 20 patients with metastatic melanoma who, after a lymphodepleting single dose of intravenous cyclophosphamide, received the infusion of tumor-infiltrating lymphocytes (TILs) and IL-2. Responses were observed in more than half of the patients and in some cases lasted longer than one year [99]. However, since TILs cannot be detected in every type of cancer, other researchers have explored the possibility of arming T-cells with a specific tumor-

directed TCR. This strategy, unfortunately, yielded disappointing results with objective responses observed in just 13% (4/31) of patients in a study published in 2006 by Morgan and coworkers [100]. In fact, several limitations to this particular strategy must be highlighted. First of all, TCRs only recognize peptide antigens exposed on the surface of an antigen-presenting cell (APC) through the MHC. Secondly, T-cells are only fully activated when a concomitant co-stimulatory bond takes place between the CD28 molecule expressed on their own surface and B7.1 and B7.2 displayed on the membrane of APCs. Like I said earlier, however, the down-regulation of MHC and co-stimulatory molecules is exactly one of the main mechanisms of immune evasion prompted by the neoplastic clone. Evidently, the dependence of TCR-mediated immune response on these elements makes it a rather inefficient ACT strategy. Moreover, since TCRs have a tendency to cross react with endogenous antigens, patients would be at high risk of developing autoimmune complications [101].

At the end of the '80, Gross and coworkers demonstrated the possibility of building a hybrid receptor, part Ig and part TCR, that could be transduced into a T-lymphocyte and eventually exposed on its surface [102]. This peculiar, single polypeptide chain construct, termed chimeric antigen receptor (CAR), combines the advantages of antibody recognition with the homing, tissue penetration and cell destruction properties of T-lymphocytes [101–103].

The antibody-based antigen recognition of CARs implies significant advantages: Ig can recognize a wide variety of antigens, not only peptides but also lipidic antigens, carbohydrates and inorganic molecules; the high-affinity antibody-antigen bond is MHC-independent and does not require the presence of membrane co-receptors [101]. Engineered T-cells harbouring the CAR construct are termed CAR T-cells.

The CAR is comprised of three domains [6,101,103,104] (fig. 1):

1. Extracellular antigen-binding domain, represented by the single-chain variable fragment (scFv) of a monoclonal antibody that recognizes a specific antigen. It contains the variable regions of the heavy (VH) and light (VL) chains of an Ig, linked sequentially by a flexible synthetic peptide.

2. Transmembrane domain, connecting the antibody moiety of the receptor to the intracellular TCR-derived part and also anchoring the CAR to the cytoplasmic membrane of the cell. It derives from a CD28 or CD8 molecule and has the structure of a hydrophobic α-helix.
3. Cytoplasmic (intracellular) domain, comprised of an activation unit (usually a CD3ζ chain) and one or more costimulatory units, which are crucial for the full activation of T-cells and whose lack was responsible for the limited activity and persistence of first-generation CAR T-cells. The most commonly used costimulatory domains are CD28 and 4-1BB (CD137) that, in particular, can be found in the CAR T-cell products employed for the treatment of B-cell lymphomas. The composition of the intracellular domain distinguishes the different generations of CARs. The first-generation CARs only contained one signaling domain, in particular the CD3ζ chain, but it was soon understood that, in order to prevent the activation-induced death and anergy of the engineered T-cells it was necessary to add a costimulatory domain. CD28 and 4-1BB are costimulatory molecules belonging to the tumor necrosis factor (TNF)-receptor family and they normally engage their ligands on the surface of APCs whenever the TCR binds to cognate antigen. Since cancer cells often lack the expression of such ligands, the fusion of CD28 or 4-1BB to CD3ζ is a brilliant way of overcoming this limitation and allowing for the full activation of the CAR T-cells, which would otherwise be impaired. Third and further generation CARs are equipped with more than one costimulatory moieties in their cytoplasmic domain.

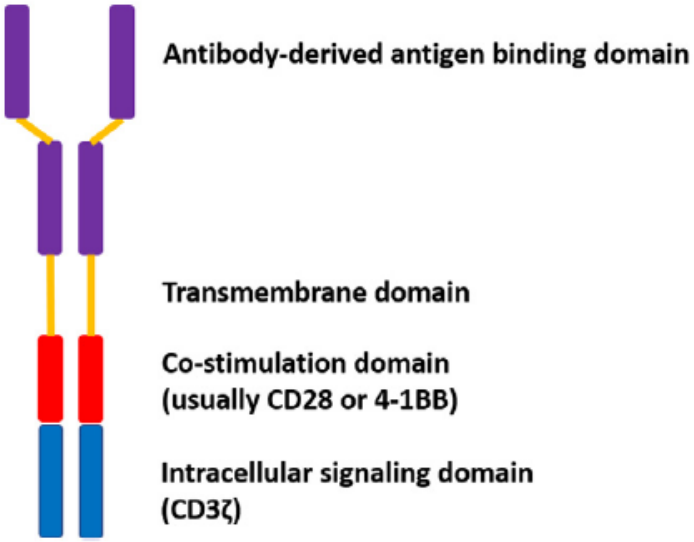

Fig. 1. Structure of a second-generation CAR.

After binding with cognate antigen on the tumor cell surface, CAR structures cluster on the engineered T-cells leading to the phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) of the signaling moiety. The downstream signaling cascade is consequently initiated, and this produces T-cell amplification, cytokine secretion and cytolytic activity of the CAR T-cell toward the target tumor cell. When armed with a CAR, CD4+ T-cells can join the cytolytic activity of CD8+ lymphocytes and release perforin and granzyme B, thus producing a direct cytotoxic effect on the target neoplastic cells [101,105].

2.2. CAR T-CELLS IN THE TREATMENT OF B-CELL LYMPHOMAS

In order to be effective and safe, CAR T-cells must target an antigen that is both highly and selectively expressed on malignant cells and absent on normal tissues, so as to prevent on-target off-tumor side effects [104].

The transmembrane glycoprotein CD19 emerged as an optimal candidate because of its uniform expression on B-cells at all stages of their differentiation and of its persistence during malignant transformation. CD19 is essential to B-cell function, since it is involved in regulating their activation in an antigen–receptor-dependent manner. Its expression is demonstrated in over 95% of B-cell malignancies; despite

its concomitant presence on normal, non-malignant B-lymphocytes, the depletion of B-cell levels and humoral immunity is already a well-known side effect of immunochemotherapy that patients can survive and that can be managed with Ig replacement [103,104].

The CAR T-cell manufacturing process is rather complex and time-consuming. Firstly, autologous T-lymphocytes are collected from the patient's peripheral bloodstream through the procedure of leukapheresis and are immediately transferred to a manufacturing facility, where they undergo genetic modification. The CAR gene is transduced into the T-cells through a viral vector, usually a replication-defective virus (lentivirus or retrovirus), which integrates the gene permanently into the cells' genome. At this point, CAR T-cells are expanded and activated *in vitro* and can subsequently be returned to the patient to be infused [103]. CAR T-cell infusion is preceded by a 3-day course of lymphodepleting chemotherapy (LC), usually containing cyclophosphamide and fludarabine, which has multiple purposes: the depletion of normal lymphocytes creates an environment that is rich in cytokines promoting CAR T-cell proliferation and activation *in vivo*; it also decreases immunosuppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, that may impair CAR T-cell proliferation and function; lastly, LC may have a minor role in decreasing the tumor burden, however unlikely this seems in chemorefractory patients [104]. Seen the considerable time passing between leukapheresis and CAR T-cell infusion, usually 30-40 days at least, a bridging therapy (BT) can be administered to patients with rapidly progressing disease in order to provide symptom control and reduction of tumor burden [103].

Following successful pivotal trials, the United States Food and Drug Administration (FDA) has approved three second-generation anti-CD19 CAR T-cell products for the treatment of LBCL: axicabtagene ciloleucel (axi-cel), tisagenlecleucel (tisa-cel) and lisocabtagene maraleucel (liso-cel) [7,8,106,107]. Axi-cel contains a CD28 costimulatory domain, whereas tisa-cel and liso-cel contain a 4-1BB costimulatory domain. Liso-cel differs from the other two products because its controlled manufacturing process leads to a predefined cellular composition and

to the administration of a fixed CD4:CD8 CAR T-cell ratio, which is associated with lower rates of toxicity [103].

Shortly after FDA approval, axi-cel and tisa-cel were also approved by the Italian Medicines Agency. Liso-cel has recently received AIFA approval, as well; the product, however, is not included on AIFA reimbursement list yet, therefore it cannot be employed in clinical practice. The current indications for utilization of axi-cel and tisa-cel in B-cell lymphomas according to AIFA reimbursement criteria (which have been continuously updated over time) are as follows:

1. Axi-cel (Yescarta®, Kite-Gilead): adult patients with DLBCL, including transformed FL (tFL) and tMZL, and patients with HGBL who are refractory to frontline immunochemotherapy or relapse within 12 months from its completion [108,109]; adult patients with DLBCL and PMBCL who are R/R after 2 or more lines of systemic therapy [7,110]; adult patients with FL who are R/R after 3 or more lines systemic therapy [111].
2. Tisa-cel (Kymriah®, Novartis): adult patients with DLBCL who are R/R after 2 or more lines of systemic therapy [8]; adult patients with FL who are R/R after 2 or more lines of systemic therapy [112].

The July 2022 update of reimbursement criteria extended the eligibility to patients aged 71-75 years and to those who previously received an allogeneic SCT, provided that at least 1 year passes between the two procedures and that transplanted patients do not have active graft-versus-host-disease (GVHD) or ongoing immunosuppressive therapy. Moreover, patients with previously known CNS involvement are no longer excluded from CAR T-cell treatment, but they must prove negative for such localization of the disease at the time of leukapheresis: they must receive 2 lumbar punctures, which have to be negative for neoplastic cells, and undergo brain imaging documenting the absence of lymphomatous lesions.

More recently, FL was included in CAR T-cells indications and, notably, so were patients with disease refractory to frontline immunochemotherapy; however, these indication are not included on AIFA reimbursement list yet.

I will herein concentrate on axi-cel and tisa-cel because, having been the first AIFA-approved CAR T-cell products, they are the only ones that we were able to employed at our Institute since August 2019, when we first started treating patients with this innovative approach.

The trial that led to the approval of axi-cel was the ZUMA-1 study, which reported impressive results in a population of high-risk, chemorefractory and heavily pretreated patients. The lymphodepleting regimen implied the administration of cyclophosphamide 500 mg/m^2 and fludarabine 30 mg/m^2 for 3 consecutive days, followed by the infusion of axi-cel at a dose of 1–2 × 10^6 CAR T-cells/kg. No BT was allowed [7]. Among the 101 infused patients, who had a diagnosis of DLBCL (*de novo* or tFL) or PMBCL, the best ORR and CRR were 83% and 58%, respectively. With a median follow-up of 15.4 months, the median PFS was 5.8 months and 42% of the patients had ongoing remission; OS at 12 months was 59% [7]. Median OS was not reached after a median follow-up period of 27.1 months, when 39% of patients were still in ongoing response [106]. At a median follow-up of 63.1 months, the median DOR was 11.1 months while the median DOCR was 62.2 months; in particular, 30 patients (30%) had an ongoing CR at data cutoff. Among the 59 patients who achieved a CR, 37 (62.7%) obtained it by the week-4 assessment, while the remaining 22 patients reached it after that time point. The median EFS and PFS were 5.7 months and 5.9 months, respectively, while the estimated 5-year EFS and PFS were 30.3% and 31.8%, respectively. Median OS among the 101 treated patients was reached at 25.8 months and the 5-year OS rate was 42.6% [110]. Regarding safety, CRS occurred in 94 patients (93%), and was grade ≥ 3 in 11 patients (11%). ICANS events occurred in 65 patients (64%) and approximately half of them were grade ≥ 3 (30 patients, 30%).

Tisa-cel was approved following the results of the phase 2 JULIET trial, which enrolled 167 patients with R/R DLBCL (including tFL) of whom 115 received infusion of the CAR T-cell product. Fifty patients (55%) had refractory disease 54 of them (49%) had prior ASCT. Bridging chemotherapy was allowed and was administered to 104 (90%) patients due to rapidly progressing disease. The conditioning LC could follow two different regimens: fludarabine 25 mg/m^2 and

cyclophosphamide 250 mg/m^2 for 3 consecutive days, or alternatively bendamustine 90 mg/m^2 administered for 2 consecutive days [8,113]. At a median follow-up of 40.3 months, the ORR was 53%, with 45 (39%) patients having a CR as their best overall response; the rate of conversion from a PR to a CR was 54%. The 3-year EFS was 78.8% among patients who maintained a CR at 3 months, and 86.5% among patients whose CR lasted up to 6 months. The median PFS was not reached for patients who had a CR at 3 months or 6 months. The median DOR in responders was not reached. The median OS for all patients and CR patients was 11.1 months and not reached, respectively [113]. CRS occurred in 66 (57%) patients and was grade ≥ 3 in 23% of cases, with a median time to resolution of 7 days (range, 5 – 9); neurological events were registered in 23 (20%) patients, were grade ≥ 3 in 11% of cases and resolved in a median time of 13 days (5 – 36) [113].

3. OUR REAL-LIFE EXPERIENCE WITH CAR T-CELLS IN LBCL

At the Haematology Institute "L. e A. Seràgnoli" in Bologna, where I am conducting my clinical and scientific activity, we started using CAR T-cell therapy on R/R LBCL patients in August 2019. I herein presents the results concerning the first 51 patients that underwent this treatment strategy from August 2019 to December 2021. Preliminary data on the first 30 treated patients, published in 2021, represented the first real-life experience with commercial CAR T-cells ever reported by an Italian centre [11].

3.1. PATIENTS AND METHODS

Between August 2019 and December 2021 we collected data on all consecutive patients with a diagnosis of LBCL that were referred to CAR T-cell therapy at our Institute. Our study is both retrospective and prospective in nature and is currently ongoing, therefore more extensive results and more thorough analysis are expected as our population increases. In accordance with the Declaration of Helsinki, all participants gave written informed consent (whenever applicable) to the collection of their data; the study was approved by the ethical committee CE AVEC of Bologna (095/2020/Oss/AOUBO).

The inclusion criteria of our study had to follow AIFA reimbursement criteria. Consequently, in order to be eligible, our patients had to have an age between 18 and 70 years old, a diagnosis of LBCL (DLBCL, either *de novo* or transformed from FL or MZL, or PMBCL), a good Eastern Cooperative Oncology Group (ECOG) performance status (0-1), an adequate organ function and had to be R/R after at least two previous lines of therapy.

The choice of using axi-cel or tisa-cel was based on the histologic subtype of the disease (specifically, patients with PMBCL can only receive axi-cel) and on the availability of manufacturing slots.

Before leukapheresis, patients were tested for HBV, HCV, HIV and *Treponema pallidum* serology; they also underwent a complete blood count, bloodwork for coagulation parameters and blood type, chest radiography, electrocardiography and cardiac ultrasound. The leukapheresis procedure was performed at the Transfusion Centre of our Hospital. Afterwards, the patients' T-cells were shipped to the manufacturing facilities across the United States and Europe, were they underwent viral transduction ad *in vitro* expansion. This process takes up approximately a month, therefore BT was administered in the meantime to patients with rapidly progressive malignancies in order to provide disease control.

Before CAR T-cell infusion, all patients were restaged with a PET/CT scan that served as a baseline disease assessment. They also had to undergo several other preliminary tests: an evaluation of cardiac and respiratory functions; a neurologic assessment comprised of a cerebral magnetic resonance imaging (MRI) scan, the administration of an electroencephalogram and neuropsychological tests; extensive bloodwork for hepatic and renal function and for baseline levels of lymphocyte subpopulations, immunoglobulins and inflammatory markers, in particular C-reactive protein (CPR), erythrocyte sedimentation rate (ESR), triglycerides, ferritin, IL-6 and IL-10. Additionally, as per institutional guidelines, all patients were started on antiepileptic prophylaxis with levetiracetam approximately 2 weeks before CAR T-cell infusion (although data regarding its usefulness are hardly conclusive [114,115]).

Once the CAR T-cell product returned to our Centre and the quality release became available, patients were admitted to our ward: chemotherapy conditioning and subsequent CAR T-cell infusion were delivered through a central venous catheter in an inpatient setting, in order to guarantee a close monitoring of AEs. All patients received LC with fludarabine and cyclophosphamide (FC) for 3 consecutive days (fludarabine: 25-30 mg/m2 and cyclophosphamide: 250-500 mg/m2) and, 2 days later, axi-cel or tisa-cel were infused. Hospitalization lasted for at least 14 days following the infusion of CAR T-cells, during which time a close monitoring of blood tests was carried out and patients were observed for the potential development of AEs.

The most common CAR T-cell-specific AEs, CRS and ICANS, were graded according to the American Society for Transplantation and Cellular Therapy (ASTCT) criteria [64]. For the recording of other AEs, we used the Common Terminology Criteria for AEs (CTCAE) version 5.0. Disease evaluation after CAR-T cell infusion was scheduled at 1, 3, 6, 12, 18, and 24 months after infusion. The imaging reports are based on the Lugano recommendations for response assessment [24].

For the safety analysis, we took into account all the patients who received the cellular infusion; for the efficacy analysis, we considered the patients who underwent at least the first disease reassessment at 1 month after CAR T-cell infusion.

3.2. OBJECTIVES OF THE STUDY

The primary aim of the study was to evaluate the ORR of our patient population, defined as the sum of CRs and PRs. The secondary end points were the following: incidence and type of AEs and serious AEs; PFS, defined as the time from infusion of the cellular product for all treated patients to the first observation of progressive disease or death as a result of any cause; DFS, estimated from the date of first documented CR to the last follow-up, or to the date of disease recurrence or death because of lymphoma or acute toxicity of study treatment; OS, calculated from the date of infusion until the time of death from any cause or last follow-up.

The survival outcomes were calculated with the Kaplan Meier method. The statistical analysis were operated through the Stata 11 software (StataCorp LP, TX).

3.3. RESULTS

3.3.1 PATIENT'S CHARACTERISTICS

Overall, 67 LBCL patients performed the leukapheresis during the period of data collection, but only 53 of them (79%) had received the cellular reinfusion by the time of data cut-off. Of the patients who did not receive the product infusion, 7 had undetectable disease at reassessment after BT, 5 had PD with rapidly deteriorating clinical conditions, 2 had SARS-CoV2 infection, 1 was diagnosed with a low-grade cerebral lesion of the neuroglia on the pre-LD MRI scan and another patient developed a neurodegenerative condition and died.

Fifty patients had performed the 1-month restaging PET-scan, thus being evaluable for efficacy. The safety analysis, on the other hand, was performed on 51 patients, because one of them (with PMBCL) received the product reinfusion but died within the first month of observation, therefore being evaluable for safety but not for efficacy; one patient, lastly, had received the infusion only a few days before data cut-off and was not included into the analysis.

One patient received an out-of-specification tisa-cel product due to an excess of interferon γ (IFNγ) and was only infused after thorough discussion and consultation with the legal department of our Hospital; he also had to sign an additional informed consent prior to undergoing the procedure.

Most patients had a diagnosis of DLBCL (44 patients), among whom were 6 individuals with tFL and 1 with tMZL; 7 patients had PMBCL. Median age at leukapheresis was 58 years (range 20-70) and 37 patients (72.5%) were males. The majority of patients had advanced-stage disease (stage III/IV in 64.7% cases) and 51% of them had bulky lesions (> 7 cm) at the time of apheresis. The median number of previous lines of therapy was 2 (range 2-7) and 94% of patients were refractory to the last treatment before CAR T-cells. Thirteen patients had already received and failed ASCT (25.5%). Thirty-nine patients (76.5%) received BT before infusion, mainly chemotherapy (51%), corticosteroids (25.6%), immunotherapy (18%) or radiotherapy (18%). Among them, 5 obtained a PR, 7 had SD and 27 had PD. The median time from apheresis to infusion was 48 days (range 29-123). Thirty-one patients (60.8%) received axi-cel while 20 of them (39.2%) received tisa-cel. Median follow-up from CAR T-cell infusion was 6.3 months.

The baseline characteristics of patients are listed in table 1:

Evaluable patients, N	51
Male/Female, N	37/14
Age in years, median (range)	58 (20 – 70)
ECOG < 1, N (%)	37 (72.5)
Stage III/IV, N (%)	33 (64.7)
B symptoms, N (%)	10 (19.6)
Bulky disease (> 7 cm), N (%)	26 (51)
LDH > UNL, N (%)	26 (50.9)
IPI ≥ 2, N (%)	22 (73.3)
≥ 1 extranodal site of disease, N (%)	36 (70.6)
Histology, N (%) DLBCL, NOS tFL tMZL PMBCL	 44 (86.3) 6 (11.8) 1 (2) 7 (13.7)
Ki67 > 50%, N (%)	37 (72.5)
Previous lines of therapy, median (range)	2 (2 – 7)
Previous ASCT, N (%)	13 (25.5)
Refractory to most recent therapy, N (%)	48 (94)
Bridging therapy, N (%) Chemotherapy (+/- rituximab) Corticosteroids Radiotherapy Immunotherapy	39 (76.5) 20 10 7 7
Median time from leukapheresis to infusion, days (range)	48 (29 – 123)

Table 1. Patients' characteristics.

3.3.2 EFFICACY

Fifty patients received the first disease reassessment 1 month from infusion. Thirty-three (66%) of them obtained a clinical response with 19 (38%) CRs and 14 (28%) PRs. Seven patients (14%) had stable disease (SD) and 10 (20%) of them experienced progressive disease (PD). Interestingly, all 6 evaluable PMBCL patients had an objective response (ORR 100%), with 4 of them obtaining a CR. Among other histologies (DLBCLs, tFL and tMZL), ORR was 61.4% (15 CRs and 12 PRs) with 16% patients having a SD and 22.7% experiencing PD.

At the 3-month time point, 47 patients were evaluable for efficacy and ORR was 57.4% (22 CRs and 5 PRs), while we registered 1 SD and 9 PDs (19%). Notably, 5 patients converted a previous PR into a CR and 1 SD improved to a CR. Four patients progressed from a PR and 4 from a SD, respectively, while only 1 patient progressed from a previous CR. One patient with PMBCL converted a previous PR into a CR.

Forty-two patients had received the 6-month disease reassessment at the time of data-cut off. Seventeen of them displayed an objective response, ORR being 40.5%. In particular, 17 patients achieved a CR (40.5%), 2 of whom with a previous PR (one of them with a diagnosis of PMBCL). One patient had SD and 5 patients had PD (2 from previous PRs and 3 from previous CRs). Specifically, one of the PMBCL patients experienced PD 6 months after infusion (relapsing from a previous CR).

At the 12-month time point, 37 patients could be assessed for efficacy. Results show 12 CRs (32.4%) and 1 PD (represented by a relapse from a CR). ORR was, therefore, 32.4%. Among the CR patients, 5 improved a previous PR within the 6^{th} month of observation; 7 patients, on the other hand, had a long-lasting CR that was first attested 1 month after infusion. Overall, the PD rate at 12 months is 67.5% (25 patients in total). Among the PMBCL group, 5 patients were alive and in CR 1 year after treatment.

3.3.3 SAFETY

Among the 51 patients infused, 42 (82.4%) developed a CRS of any grade; in most cases, CRS was grade ≤ 2 (in particular, grade 1 in 54.9% of patients), with only 5/51 patients (9.8%) experiencing grade > 3 CRS. While for most patients the CRS started as grade 1, two patients experienced a grade 2 onset of the event. The median time to onset was 2 days (range 1 – 11), while the median time to resolution was 4 days (range 1 – 33).

Seventeen patients (33.3%) developed neurotoxicity, with 7 of them experiencing grade ≥ 3 ICANS (13.5%). In all patients, ICANS was preceded by a CRS event. The median time to onset was 5 days (range 1 – 12) and the median time to recovery was 8.5 days (range 2 – 53) for ICANS.

Twenty-eight patients (54.9%) received tocilizumab for CRS, with a median number of 3 doses (range 1 – 4). Among these patients, 19 (68%) were also treated with steroids due to refractoriness to tocilizumab (5 patients) or development of grade ≥ 2 ICANS (14 patients). In total, 23 patients received steroids (45%). Ten patients (23.3%) required admission to the Intensive Care Unit (ICU); 8 of them required at least one dose of siltuximab in addition to tocilizumab and/or corticosteroids, while 2 patients also needed the administration of anakinra due to refractoriness of CRS and ICANS to the other treatments. One patient was diagnosed with macrophage activation syndrome at day 28 post infusion: she was started on high dose steroids with partial benefit, but subsequently died due to PD within 70 days from CAR T-cell treatment.

Concerning haematological toxicity, 47 patients (92.1%) developed neutropenia, which reached grade ≥ 3 in 46 individuals (90%); none of them, however, experienced life-threatening infectious complications that were directly related to the decrease in neutrophil count. Anemia and thrombocytopenia were less frequent (72.5% and 68.6%, respectively) and generally grade < 3. Eight patients had hypofibrinogenemia (15.7%), all of them grade ≥ 2 and requiring supportive therapy.

3.3.4 SURVIVAL

Seventeen patients (33.3%) died during the surveillance period, 11 of them because of PD (21.6%) and the remaining 6 due to complications: 2 deaths were attributed to neurotoxicity, 2 patients died because of severe acute respiratory syndrome Coronavirus 2 (SARS-CoV2)-related interstitial pneumonia, 1 patient had a septic shock and another one died as a result of an infectious complication that occurred after allogeneic SCT (performed because of PD at 3 months after CAR T-cell infusion). After a follow-up of 22.7 and 21.7 months, respectively, PFS was 27.2% and DFS was 46.7%; median PFS was reached at 6 months and median DFS at 15 months. At 24.2 months, OS was 28% with median OS reached at 16 months. The Kaplan-Meier curves are reported in the following figures (fig. 2, 3, 4):

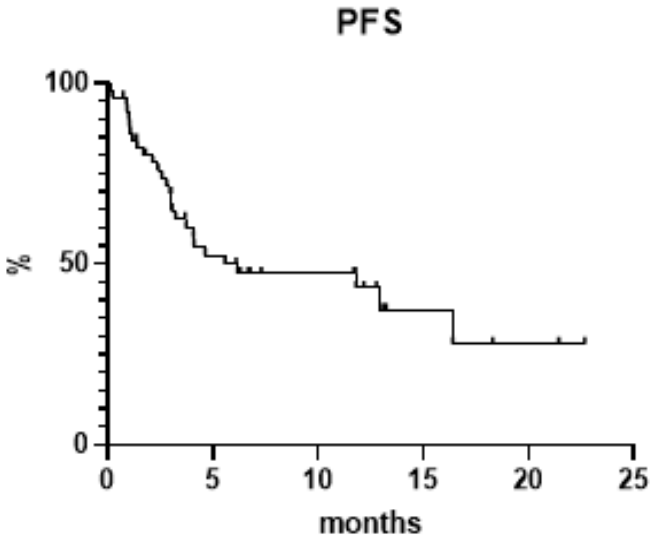

Fig. 2. PFS (27.2% at 22.7 months, median reached at 6 months).

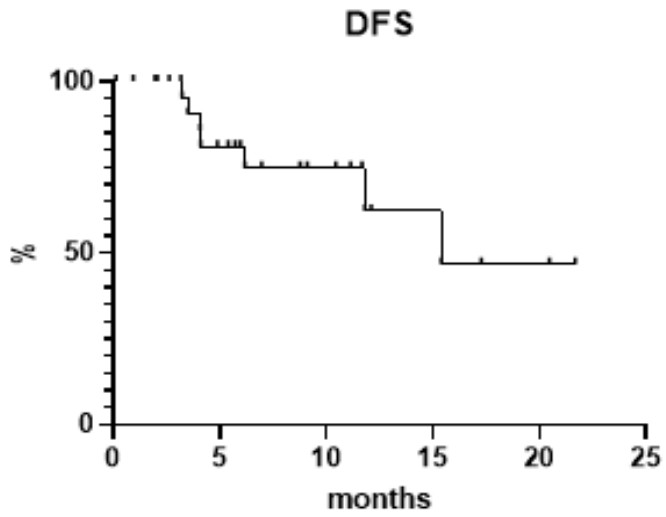

Fig. 3. DFS (46.7% at 21.7 months, median reached at 15 months).

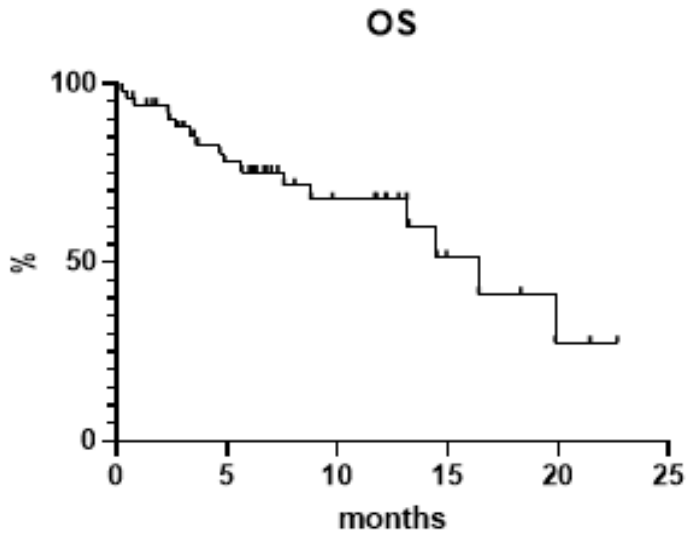

Fig. 3. OS (27.4% at 24.2 months, median reached at 16 months).

4. DISCUSSION

During these last years, the introduction of CAR T-cell therapy has revolutionized the approach to patients with R/R aggressive B-cell lymphomas, whose prognosis used to be invariably dismal. Historically, patients with DLBCL who did not respond to frontline immunochemotherapy or relapsed within 12 months from ASCT had an estimated OS of approximately 6 months [3]. Although various novel agents have been demonstrated to have clinical activity and have gradually been added to the therapeutic armamentarium of LBCL, results remain largely unsatisfactory, especially for younger patients [14].

Concerning PMBCL, it is well-known that patients who prove refractory or relapse after initial immunochemotherapy tend to be utterly chemorefractory and can hardly be salvaged through standard HCT and ASCT [72]. The rate of 2-year OS for individuals with R/R disease is set between 15% and 29% [95]. Among the most recently introduced agents, immune checkpoint inhibitors are the only ones to have provided a significant improvement in the prognosis of this high-risk population. In particular, single-agent pembrolizumab is associated with 4-year PFS and OS rates of 33% and 45.3%, respectively, while the combination of nivolumab and brentuximab vedotin yields a 2-year PFS rate of 55.5% with an OS rate of 75.5% [92,95].

Within this scenario, the advent of CAR T-cells has been nothing short of game-changing. The response rates observed with this treatment approach, as well as response durability and survival outcomes, are absolutely unprecedented and led CAR T-cells to become the new standard of care for patients with LBCL whose disease is R/R after 2 or more lines of therapy [116]. Compared to the outcomes of LBCL patients treated with conventional chemotherapy in the SCHOLAR-1 study, for instance, the ZUMA-1 trial reported a 73% reduction in the risk of death with a 2-year survival rate of 54% (versus 20% in SCHOLAR-1) [117].

Following the excellent results of pivotal trials [7,8,107], the first three anti-CD19 CAR T-cell products axi-cel, tisa-cel and liso-cel have received regulatory approval both in the US and Europe. Along with extraordinary results, however, this treatment strategy is characterized by a peculiar toxicity profile that requires

appropriate management and a careful selection of patients [9]. Therefore, it has quickly become clear that extensive reports on the real-life employment of CAR T-cells were crucial to deepen our knowledge of short- and long-term AEs, to deal with emerging mechanisms of resistance, to optimize the selection of patients outside of clinical trials criteria and, on the whole, to understand whether results are reproducible in populations with diverse characteristics [10,118].

One of the most useful aspects of real-world studies is represented by the treatment of patients who would have been ineligible for pivotal trials on CAR T-cells. Some studies have already demonstrated that high response rates can be obtained even on patients who would not have met the inclusion criteria for the ZUMA-1 or JULIET trials due to advanced age or comorbidity load [119–121]. In particular, Jacobson and coworkers found that, while ORR with axi-cel was not affected by different eligibility criteria, CRR, DOR and survival were inferior in ineligible patients compared to ZUMA-1. Moreover, the incidence of CRS and ICANS was similar between this real-world population and the pivotal trial [119]. Several experiences point out that, whereas it is true that patients do not necessarily need to meet the trials eligibility criteria to benefit from CAR T-cells and that advanced age should no longer be considered an issue, individuals with poor ECOG PS tend to have a less favourable outcome [120,122–126]. The reimbursement conditions for CAR T-cells prescription established by AIFA have been modelled on the eligibility criteria of the ZUMA-1 and JULIET studies; consequently, the characteristics of our patients do not differ much from those of the trials' populations. This constitutes a limitation of our study, since it prevents us from evaluating the feasibility of CAR T-cell therapy in patients with particular features such as a higher comorbidity burden, the presence of CNS disease, a HIV-positive status, age older than 75 years or ECOG PS > 2.

Presently, little data exist regarding a direct comparison of the efficacy and safety of axi-cel and tisa-cel, except for some matching-adjusted indirect comparisons (MAICs) [118,126–132]. Clearly, comparing the results of clinical trials is inappropriate due to large differences between study designs, such as the patients' characteristics, the possibility of giving BT for disease control during the manufacturing process

and the different LC regimens employed. In 2022, Bachy and coworkers analysed the outcomes of 809 patients with R/R DLBCL treated with commercial axi-cel or tisa-cel and included in the DESCAR-T registry [118]. The best ORR and CRR were found to be 80% and 60% for patients treated with axi-cel versus 66% and 42% for tisa-cel, which is consistent with the results reported in the clinical trials [118]. After a median follow-up of 11.7 months, the 1-year PFS was 46.5% and 33.2% for axi-cel and tisa-cel, respectively; similarly, 1-year OS was longer for axi-cel compared to tisa-cel (63.5% versus 48.8%, respectively) and median OS was 11.2 months for tisa-cel while it was not reached with axi-cel. Axi-cel, on the other hand, was also associated with a higher toxicity: grade 1-2 CRS was significantly more frequent compared to tisa-cel treatment, while no substantial differences were observed regarding grade ≥ 3; however, both grade 1-2 and grade ≥ 3 ICANS events were more common with axi-cel than with tisa-cel [118]. Our study includes both patients treated with axi-cel and with tisa-cel, therefore once we have collected a larger number of cases we might be able to analyse the differences in outcome and toxicity associated with the two products.

Several studies have shown that pre-infusion tumour burden is associated with increased incidence and severity of CRS and ICANS, as well as with outcomes after CAR T-cell therapy [133–135]. In particular, published data provide preliminary evidence that CAR T-cell products maintain their *in vivo* expansion potential even in the absence of measurable disease, probably due to their ability to respond to residual lymphomatous cells that cannot be detected by standard imaging techniques [133]. In fact, Bishop and coworkers reported the peak CAR T-cell expansion and toxicities in 7 patients receiving tisa-cel infusion within the JULIET trial while in CMR, and pointed out that results were comparable to those observed in patients with measurable disease [133]. A real-world experience published by Wudhikarn in 2022, on the other hand, described a significantly lower incidence of CRS and ICANS in 33 patients with no residual lymphoma at the time of CAR T-cell therapy (36.3% and 6.1%, respectively), with no grade ≥ 3 CRS events and one patient experiencing grade 4 ICANS. Furthermore, these disease-free patients appeared to have superior survival compared to those with persistent lymphoma at

the time of cellular infusion [135]. In our study, patients received a disease reassessment both at leukapheresis and prior to LC, which means both before and after undergoing BT. In the future, this will allow us to specifically evaluate the impact of disease status and tumour burden after BT on the response to CAR T-cell treatment, as well as on toxicity and long-term survival. The unavailability of these data represents a limitation of the present analysis, but we plan on exploring this topic further as our study population expands. Currently, AIFA contemplates reimbursement of CAR T-cell treatment only for patients with detectable disease; however, available data suggest that CAR T-cells may be able to eradicate chemorefractory subclones of LBCL that escape salvage HDC even in individuals with negative FDG-PET scans. Prospective studies exploring the possibility of giving CAR T-cell therapy as a consolidation strategy in patients with undetectable disease could eventually shed light on this interesting aspect [135].

Another intriguing topic is represented by the determinants of resistance to CAR T-cells that drive disease relapse. Available data indicate that resistance mechanisms are multifaceted and depend both on the target and effector cells [136]. The down-regulation or loss of CD19 on the surface of neoplastic cells is a well-established mechanisms that lymphoma employs in order to escape from engineered T-cells or other CD19-directed agents. In addition, it has been observed that numerous alterations can occur in genes associated with B-cell identity, immune checkpoints and tumour microenvironment [136]. This issue clearly warrants further elucidation through large, prospective studies. Whenever safe, we try to perform biopsies on lymphoma lesions of patients who relapse after CAR T-cell treatment in order to obtain as much information as possible regarding the tumour characteristics and, consequently, the most appropriate salvage regimen.

Overall, despite the small sample size and relatively short follow-up, our results confirm that CAR T-cells retain their effectiveness and safety even in a non-trial setting. The incidence of CRS and ICANS was consistent both with clinical trials and real-world data, and so were the rates of grade ≥ 3 AEs [122,126,128,129,131,137,138].

5. CONCLUSIONS AND FUTURE DIRECTIONS

On the whole, our study confirms that CAR T-cell therapy is able to rescue a considerable proportion of patients with R/R DLBCL and PMBCL, whose prognosis would otherwise be rather dismal. Regarding the safety analysis, our results compare favourably with those reported in clinical trials [7,8,110,113,139] and other real-world experiences [118–120,122,123,126,128,130,140,141].

Currently, we are collecting data concerning the tracking of CAR T-cells in the peripheral blood of infused patients at specific time points, mainly corresponding to pre-defined radiologic disease reassessments, in order to correlate their expansion and persistence to response and survival.

A larger population will also allow us to analyse the impact of different BT approaches. Existing data regarding this specific issue are conflicting: some authors report that requiring BT is in itself an unfavourable prognostic feature [141], while others failed to observe this association although reporting higher rates of haematological toxicity in individuals exposed to BT [142]. However, it should be noted that specific BT strategies seem to yield the best outcomes: RT and polatuzumab-based systemic therapies, for example, are associated with particularly good responses [141–143].

Our study was the first real-life Italian experience with CAR T-cells to be published in 2021 [11] and, being an ongoing, prospective study, it is constantly increasing in terms of sample size and depth of analysis. In the next years we will update our results and we will likely be able to explore different aspects of this innovative and paradigm-shifting treatment strategy.

The analysis I herein presented was performed on the 51 patients who received the CAR T-cell infusion at our Institute. The updated analysis that we are currently conducting on a larger population, which encompasses the individuals treated since December 2021, will also include an intention-to-treat analysis performed on all the patients referred to this treatment approach, regardless of whether they actually proceeded to cellular infusion or not. The acquisition of these data, the lack of which represents an important limitation of the present work, will help us understand which patient and/or disease features hinder the completion of the

treatment plan and, possibly, how to optimize the procedures in order to allow the greatest number of patients to benefit from CAR T-cell therapy [140].

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