Received: 2 June 2023 | Revised: 10 July 2023 | Accepted: 10 July 2023

DOI: 10.1111/ejh.14052

REVIEW

European Journal of Haematology

WILEY

Hematopoiesis and immune reconstitution after CD19 directed chimeric antigen receptor T-cells (CAR-T): A comprehensive review on incidence, risk factors and current management

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Abstract

Impaired function of hematopoiesis after treatment with chimeric antigen T-cells (CAR-T) is a frequent finding and can interest a wide range of patients, regardless of age and underlying disease. Trilinear cytopenias, as well as hypogammaglobulinemia, B-cell aplasia, and T-cell impairment, can severely affect the infectious risk of CAR-T recipients, as well as their quality of life. In this review, we provide an overview of defects in hematopoiesis after CAR-T, starting with a summary of different definitions and thresholds. We then move to summarize the main pathogenetic mechanisms of cytopenias, and we offer insight into cytomorphological aspects, the role of clonal hematopoiesis, and the risk of secondary myeloid malignancies. Subsequently, we expose the major findings and reports on T-cell and B-cell quantitative and functional impairment after CAR-T. Finally, we provide an overview of current recommendations and leading experiences regarding the management of cytopenias and defective B- and T-cell function.

KEYWORDS
CAR-T cells, cytopenia, hematopoiesis, hypogammaglobulinemia

Novelty statements

What is the new aspect of your work?

From the beginning of routine use of CAR-T cells, physicians have realized they were facing a new phenomenon while approaching post-CAR-T hematopoiesis, due to its peculiar kinetics, pathophysiology, and management. During the first 5 years of the "CAR-T" era, most research groups have produced their own definitions and predictive scores for describing impaired hematopoiesis after CAR-T, and a full harmonization with specific guidelines is still needed.

What is the central finding of your work?

In this review, we take stock of current knowledge with respect to this issue. We initially provide an overview of the definitions and threshold values adopted in the various studies and describe the incidence of cytopenias and hypogammaglobulinemia in the various papers, providing summary tables. Next, we summarize the various pathogenetic mechanisms underlying deficient hematopoiesis after CAR-T, and describe the role of CHIP and the incidence of secondary myeloid neoplasms, again providing summary tables. In a subsequent section, we focus on T and B lymphocyte function post CAR-T, and conclude with an in-depth discussion of the management of hematologic toxicities recommended in the various papers.

What is (or could be) the specific clinical relevance of your work?

We feel that this review can help clarify the numerous and uneven contributions on this important topic, and provide a useful tool for having an overall view with the aim of supporting in understanding and every-day managing CAR-T cells.

1 | INTRODUCTION

Chimeric antigen receptor T-cell (CAR-T) directed against the CD19 antigen is emerging as a highly effective treatment for many relapsed or refractory lymphoproliferative disorders. As cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) have been widely described as acute toxicities, the high impact of cytopenias on infections, bleedings, and nonrelapse mortality and morbidity is gaining increased attention.[1,2] The CD19-directed CAR-T products most widely used are tisagenlecleucel (tisa-cel), axicabtagene ciloleucel (axi-cel), brexucabtagene autoleucel (brexu-cel) and lisocabtagene maraleucel (liso-cel). In this review, we provide an update for 2023 on the state of art of cytopenia occurring after CAR-T.

1.1 | Definitions

Cytopenias occurring after treatment with CAR-T have been described since 2019, with a case series of bone marrow failure syndromes reported after CAR-T with or without co-existing clonal myelodysplastic syndrome. That phenomenon was first named "persistent cytopenias after T-cell therapy (PCTT)".[3] Similar findings of unexplained cytopenias with a variable depth and a mostly biphasic incidence have subsequently been reported and better described in larger series, with the name of CAR-T-OPENIA[4] or Immune Effector Cell-Associated Hematotoxicity (ICAHT).[5]

Cytopenias are normally graded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

Grade 3 (G3) and 4 (G4) neutropenia occur when absolute neutrophils count (ANC) is lower than 1×10^9/L and 0.5×10^9/L, respectively. Moreover, grade 4 neutropenia can be described as "severe" (ANC $<0.5 \times 10^9$/L) or "profound" (ANC $<0.1 \times 10^9$/L).

Neutropenia is defined as "protracted" when lasting at least 7 days, and "prolonged" when an ANC $<1 \times 10^9$/L is detected after 21 days from infusion of CAR-T, in the absence of subsequent therapy with myelosuppressive drugs.[6]

Neutrophil values can restore with a quick recovery (stable ANC $>1.5 \times 10^9$/L by day 21), intermittent recovery kinetics (ANC ≥1.5 with subsequent decrease below 1×10^9/L after day 21), or with an aplastic profile (ANC $<0.5 \times 10^9$/L for ≥14 days). Currently, there is no data clustering the kinetics of platelet recovery.

According to some authors, cytopenia can be categorized by the timing of occurrence and persistence into three groups: present by day 30 ("early"), between day 30 and 90 ("prolonged" or "short-term"), and over day 90 ("late"),[4,7] while a recent consensus from EHA/EBMT defined day 30 as cut-off for discriminating "early" and "late" cytopenia.[5]

1.2 | Incidence

Estimating the real incidence of cytopenia still remains a great challenge, as the lack of a homogenous definition of prolonged and severe cytopenia and the biphasic pattern[8] make it difficult to compare studies that differ in their endpoints.

After CAR-T infusion, patients experienced any grade cytopenia in 9%–97%, 6%–66.7%, and 1%–66.7% cases up to day 30, between days 30 and 90, and after day 90, respectively, according to different reports (see Table 1).

Grade 3–4 neutropenia is the most frequent cytopenia in the early setting (up to 91% of patients),[8] while deeper thrombocytopenia can be observed more frequently in the short-term (up to 66.7%),[24] as shown in Table 1.

2 | RISK FACTORS, PATHOGENESIS AND PREDICTIVE MODEL

Several reports have tried to identify risk factors able to predict cytopenia after treatment with CAR-T, and pathogenetic factors underlying the development of cytopenias are not yet completely understood. As a concept, those factors may be divided into two

TABLE 1 Incidence of grade 3–4 cytopenia according to major reports.

Reference	Patients	Target	Costimulation	Setting	Early (<day 30)			Prolonged (day 30–90)			Late (>day 90)		
					HB	PLT	ANC	HB	PLT	ANC	HB	PLT	ANC
Cordeiro[9]	86	CD19	4-1BB	CT									16%
Fried[8]	39	CD19	CD28	CT	55%	28%	71%						
Strati[10]	31	CD19	CD28	CT	16%	42%	29%						11%
Abramson[11]	269	CD19	4-1BB	CT	27%	37%	60%						
Jain[12]	83	CD19, BCMA	CD28, 4-1BB	RL	39%	49%	67%	7%	10%	19%	0%	0%	6%
Nahas[3]	22	CD19	CD28	RL						38%			
Rejeski[6]	258	CD19	CD28, 4-1BB	RL	62%	69%	91%						
Juluri[13]	173	CD19	4-1BB	CT	16%	44%	49%	21%	38%	29%	11%	13%	33%
Lievin[14]	122	CD19	CD28, 4-1BB	RL			78%	6%	24%	26%	1%	6%	13%
Zhou[15]	133	CD19, CD20, CD22	CD28, 4-1BB	RL	30%	41%	71%		35%	18%			
Jacobson[16]	148	CD19	CD28	CT			34%						
Teipel[17]	32	CD19	CD28, 4-1BB	RL		70%	63%		47%	39%			
Penack[18]	398	CD19	CD28, 4-1BB	RL			9%						12%
Iqbal[19]	88	CD19	CD28, 4-1BB	RL				66%	48%	16%	48%	48%	13%
Kitamura[20]	21	CD19	CD28, 4-1BB	RL									67%
Mullanfiroze[21]	99	CD19, CD22	4-1BB	RL			47%						
Wang[22]	109	CD19	CD28	CT	41%	65%	71%	27%	67%	53%			
Kuhnl[23]	300	CD19	CD28, 4-1BB	RL								6%	9%

Note: The incidence of Grade 3–4 cytopenia, described separately by anemia, neutropenia, or thrombocytopenia, is reported according to the most representative prospective or retrospective studies.
Abbreviations: ANC, absolute neutrophil count; CT, clinical trial; Hb, hemoglobin; PLT, platelets; Pts, patients; RL, real life.

groups: factors related to the patients, their medical history, and inherited hematopoietic stress, and factors related to the hyper-inflammatory status caused by the disease and by CAR-T infusion. Intercurrent factors with specific pathogenetic pathways will be discussed separately.

2.1 | Factors related to patients, previous treatments, and hematopoietic stress

When considering the first group, baseline cytopenias assessed at the beginning of the lymphodepleting regimen have been associated with a higher risk of early cytopenia.[3,4,15] These findings could suggest the possibility that previous treatments may have resulted in an inadequate bone marrow function in the presence of hematopoietic stress. Lymphodepletion is mainly based on fludarabine and cyclophosphamide (FC) or bendamustine. In a recent retrospective study of 133 patients, lomustine/etoposide/cytarabine-based lymphodepletion, and prior HSCT were independently associated with early thrombocytopenia, but not with late hematologic toxicity.[15] Prior bone marrow infiltration and the number of prior lines of chemotherapy and/or radiotherapy may also contribute to bone marrow dysfunction and lead to persistent cytopenias after CAR-T therapy.[8,10,12,15,25]

2.2 | Factors related to the hyper-inflammatory state

With regards to factors related to the hyper-inflammatory state, the presence of grade 3–4 CRS, a higher peak C-reactive protein, as well as spikes in ferritin and cytokine levels—especially interleukin (IL)-6—were found predictive for severe cytopenia and slower hematological recovery.[3,4,15,24] Overall, the role of inflammatory cytokines in the development of post-CAR T-cell cytopenias may result controversial. Tumor antigen recognition by engineered CARs leads to T-cell activation and proliferation, resulting in macrophage activation and release of cytokines such as IFN-γ, IL-2R, IL-6, IL-10, IL-12, IL-18, IL-1β, TNF-α, IL-33, and ferritin.[26,27] High levels of IFN-γ can trigger myelosuppression through induction of stem cell exhaustion by hematopoiesis stress, and stem cell niche damage.[28] Notably, patients infused with CAR-T tend to show similar elevated patterns of IFN-γ, IL-6, and IL-8 as patients with acquired aplastic anemia (AA) and hypocellular myelodysplastic syndrome (MDS).[29,30] This is consistent with data showing that IFN-γ may impair hematopoiesis by impeding thrombopoietin (TPO) to fully access its binding site in hematopoietic progenitors.[31] The development of CRS is associated with the release of inflammatory cytokines,[30] and a nomogram of cytokines including CX3CL1, GZMB, IL-4, IL-6 and PDGFAA may also predict severe CRS before they become clinically evident.[32] Several studies with different

products and different settings have shown a correlation between higher-grade CRS or ICANS and the development of cytopenias[8,12,13,24] despite this correlation was not always confirmed.[3,12]

The impact of differences in the CAR constructs on hematological toxicity is still unclear. These effects may be secondary to differences in expansion and persistence of the CAR-T cells themselves due to the different costimulatory domains (CD-28 for axi-cel and brexu-cel and 4-1BB for tisa-cel and liso-cel).[33] As CAR-T products differ in the risk of cytokine releases and peaks, this could be the link for associations between the design of the CAR construct with cytopenias that were significant in univariate models (milder cytopenia in tisa-cel recipients) but lost significance in multivariate analysis.[12]

2.3 | Predictive score

In 2021, Rejeski and colleagues proposed a risk score (CAR-HEMATOTOX) to identify patients at higher risk to develop prolonged cytopenia and severe infectious complications. To do so, they confirmed that baseline cytopenias, high tumor burden, and elevated levels of serum/plasma inflammatory markers were predictive for post-CAR-T cytopenia.[6] In the CAR-HEMATOTOX score, variables related both to bone-marrow previous impairment and to hyperinflammatory state were considered: baseline ANC, hemoglobin, platelet count, and inflammatory markers (C-reactive protein, ferritin) were assigned 0, 1 or 2 points according to specific thresholds. CAR-HEMATOTOX sum score of 2 or more defines a group of patients with a higher risk of prolonged and profound neutropenia and aplastic recovery pattern.

2.4 | Immune effector cell-associated HLH-like syndrome (IEC-HS)

Immune effector cell-associated HLH-like syndrome (IEC-HS) is worth to be considered separately. IEC-HS is a pathological and biochemical hyperinflammatory syndrome that (1) manifests with features of macrophage activation/HLH, (2) is attributable to IEC therapy, and (3) is associated with progression or new onset of cytopenias, hyperferritinemia, coagulopathy with hypofibrinogenemia, and/or transaminitis.[27] Macrophage activation leads to the clinical manifestation of IEC-HS reflecting the expression of an exaggerated inflammatory response following CRS or CAR-T expansion at the resolution of CRS.[34,35] The median time to onset of IEC-HS was 11.5 days (range 8–20)[34] or 14 days (range 7–25).[35] Therefore, IEC-HS is a very important clinical entity in the differential diagnosis of early cytopenias.

2.5 | Intercurrent factors: infections, autoimmune cytopenias and thrombotic microangiopathy

Infections, including bacterial, fungal, and viral reactivations, can be both the cause and the consequence of cytopenias after CAR T-cell therapy.[15,36] However, it is still debated whether prolonged neutropenia after CAR T-cell therapy is an independent risk factor for fungal and bacterial infections.[10,15,37] Myelotoxicity due to viral infections or drugs needs to be considered when assessing cytopenic patients after CAR-T. Autoimmune cytopenias and thrombotic microangiopathy may also occur after CAR T-cell therapy, resulting in at least transient cytopenias.[38,39]

3 | CYTOMORPHOLOGIC FEATURES

The cytomorphological assessment of both CAR-T cells and CAR-T exposed hematopoietic precursors can present diagnostic pitfalls.

CAR-T cells have been described as a heterogeneous lymphoid population that can morphologically resemble aggressive lymphoma cells or lymphoblasts; most commonly, they appear as small or medium-sized lymphocytes with basophilic cytoplasm and hyperchromic chromatin with characteristic nuclear indentations. Nevertheless, CAR-T can appear with promonocytoid features and immature chromatin, or they can resemble large granular lymphocytes. In some cases, CAR-T cells appear as a giant blastic population with immature chromatin, multiple nucleoli and abundant hyperbasophilic cytoplasm with hypertrophic Golgi (Figure 1).[40] These cytomorphological features can be found both in peripheral blood, bone marrow or cerebrospinal fluid[41–43]: pleomorphic appearance of CAR-T cells underline the intensity of their proliferative activation driven by their antitumoral activities. No cytomorphological aspects can help in the differential diagnosis between a pathological lymphoid population and CAR-T cells. Immunophenotyping is necessary in these cases.

In CAR-T-treated patients, bone marrow examination is indicated in case of severe persistent cytopenias. Once a secondary myeloid neoplasm has been ruled out, often a morphologically hypo-aplastic cellularity is reported.[44]

Transient dysplastic features can occur in many inflammatory circumstances: infections, drugs and chemotherapy, nutritional deficiencies, HSCT, or genetic disorders can variously be associated with transient myelodysplasia, along with reactive plasmacytosis, hemophagocytosis or even fibrosis and necrosis.[45,46] Recently, transient dysplastic features have also been described in bone marrow samples of a patient with persistent cytopenia after CAR-T: in that case, cytogenetics and NGS were not suggestive of therapy-related MDS, and the patient recovered spontaneously both from cytopenia and from cytomorphological dysplasia.[47] Cytogenetic and molecular studies should be integrated to properly diagnose secondary myeloid neoplasms.

4 | CLONAL HEMATOPOIESIS AND CAR-T

Clonal hematopoiesis of indeterminate potential (CHIP) is defined by the identification of somatic pathogenic variants in driver oncogenes, with a variant allele frequency (VAF) of at least 2%, in absence of cytopenia or bone marrow dysplasia.[48] The prevalence of clonal hemopoiesis (CH) of indeterminate potential increases with age and has been estimated to be around 20% in a healthy adult population.[49]

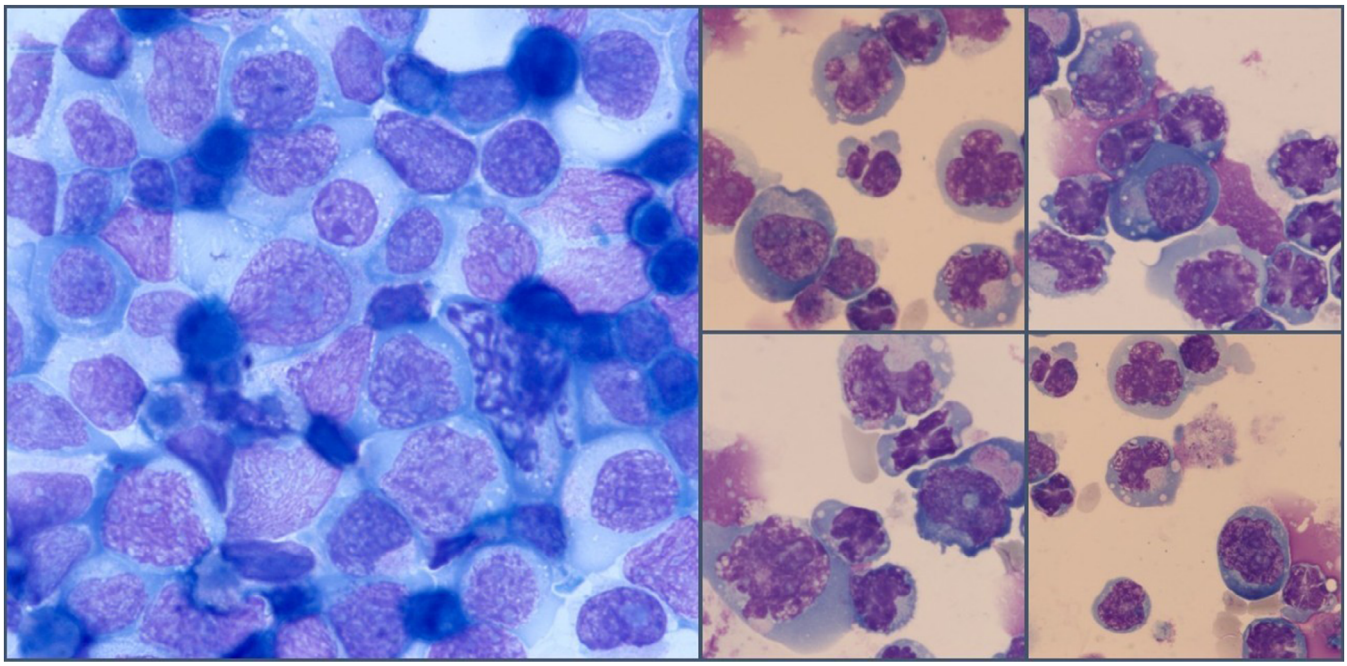

FIGURE 1 Adapted from Galli et al.[40] The figure highlights cytomorphological features pictured from CAR-T bags leftovers (see the text for full description). These features might be also found in bone marrow, peripheral blood or cerebrospinal fluid, and may require careful differential diagnosis in patients receiving CAR-T for lymphoma or acute leukemia.

CHIP is more frequent in elderly or pre-treated patients. Patients candidate to receive CAR-T therapy share a high probability to host a CHIP.[50,51] Discordant real-world data was published on how CHIP would be related to CAR-T outcomes. Miller and colleagues detected a CHIP in 48% of 154 patients with NHL and MM receiving CAR-T,[52] with predominant mutations in PPM1D, DNMT3A, TP53, TET2 and SRCAP. Among patients younger than 60 years, the presence of CHIP predicted higher probability to achieve a CR (77.6% vs. 57.9%) and to experience CRS ≥ grade 2 (77.8% vs. 45.9%); nevertheless, no differences were noticed in terms of survival or disease relapse. In the smaller cohort of 32 patients described by Teipel and colleagues,[17] CHIP was detected in 34% of cases prior to CAR-T cell therapy, mainly DNMT3A and TP53. CHIP carriers experienced better response and overall survival after CAR-T cell infusion. In this report, authors did not find an association between CHIP and CRS. Saini and colleagues, reported a preexisting CHIP in 36.8% of 114 patients with NHL being candidates for CAR-T cell therapy.[53] The most frequent mutations were PPMD1, DNMT3A, TP53, TET2 and ASXL1. Although CHIP did not significantly affect response rate or survival outcome, the experience of an ICANS of grade 3 or higher appeared to be closely related to CHIP (45.2% vs. 25%), particularly for those mutations associated with inflammation (DNMT3A, ASXL1 and TET2). Murine models with knockout of CHIP-related genes, such as DNMT3A or TET2, showed an impaired T-cell function.[54,55] CHIP might affect host-immune responses and inflammatory pathways in the tumor microenvironment in which CAR-T cells have to exert their function.[53,56] Single case reports of disruption of TET2[57] and CBL[58] during CAR-T manufacturing for CLL and ALL, respectively, described enhanced product expansion and improved response.

It is difficult to consider CAR-T therapy as causative of clonal hematopoiesis (CH), and this can be excluded when a CH already existed before CAR-T cell infusion.[17,52,53,59] Although the presence of a CH prior to CAR-T cell therapy does not appear to affect survival, it might predict increased response, higher risk for clinically significant CRS and higher risk for myeloid neoplasm in presence of TP53.[52,53,60] With regards to CHIP occurring after CAR-T cell therapy, it has been shown that patients with preexisting CHIP, may acquire new mutations or expanded their previous VAF during follow-up.[53] The cumulative incidence of t-MN at 24 months appeared significantly higher for patients with preexisting CHIP as compared to CAR-T cell recipients without prior CHIP (19% vs. 4.2%).[53]

5 | LONG-TERM ADVERSE EFFECTS ASSOCIATED WITH HEMATOPOIESIS AFTER CAR-T: SECONDARY MYELOID NEOPLASMS

There are still few reports on the incidence of myeloid neoplasms in CAR-T cells treated patients, which are now summarized in Table 2.

In the largest collection of 1297 patients with R/R LBCL receiving commercial axi-cel and registered at the Center for International Blood and Marrow Transplant Research (CIBMTR), 15 (1.1%) cases of myelodysplastic syndrome (MDS) were reported, with a median follow-up of 12.9 months.[61] A MD Anderson Cancer Center study reported the highest MDS incidence of 12.9% (4/31) from ZUMA-1 and ZUMA-9. These cases were diagnosed after a median of 13.5 months (range, 4–26 months) from axi-cel infusion. No

TABLE 2 Incidence of therapy-related myeloid neoplasms (t-MN) according to different reports.

Reference	CAR-T product	Patients	Disease	MDS	AML	Median onset from CAR-T infusion to t-MN (months)
Jacobson[61]	Axi-cel	1297	LBCL	1.1%		
Strati[10]	Axi-cel	31	LBCL (ZUMA-1 and ZUMA-9)	12.9%		13.5
Cordeiro[9]	N.A.	86	LBCL, ALL, CLL	5%		6
Chong[62]	Tisa-cel	38		3%	3%	
Wang[63]	Brexu-cel	68	MCL (ZUMA-2)	1.5%	1.5%	31
Hsieh[64]	N.A.	420	B-ALL	0.03%	0.5%	14
Zhao[65]	Local	126	LBCL	2.5%	0.8%	16.5
Miller[52]	N.A.	154	LBCL, MM	2.4%		

Note: A summary of incidence of myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) after CAR-T infusion is provided. The median time from CAR-T infusion to t-MN, as well as number of patients included in different studies and type of CAR-T product are shown, as well.

significant difference in the incidence of MDS was observed between patients who developed or did not develop grade 3–4 cytopenias at 30 days.[10] In a report from the phase 1 ZUMA-1 trial, one case of MDS with a complex karyotype and nonsynonymous DNMT3A and TP53 (double) mutations has been reported. These mutations were pre-existing to CAR-T treatment.[66]

In 83 patients treated with CAR-T for various B-cell neoplasia with 4 (range 1–7) median prior lines of therapy, four patients (5%) developed MDS.[9,27] Two of the four patients had cytogenetic abnormalities prior to CAR-T cell therapy. The median time from CAR-T cell infusion to the diagnosis of MDS was 6 months (range, 4–17).[9]

In the five-years follow-up of 38 patients treated with tisa.-cel, one patient developed acute myeloid leukemia (AML) and another patient MDS.[62]

In the ZUMA-2 study, infusing CAR-T therapy for R/R MCL, two patients (2.9%) evolved into t-MN, namely one MDS and one AML, 25.2 and 37.5 months post-infusion, respectively, after a median follow-up of 35.6 months.[63]

Based on a multicenter retrospective analysis of 420 children and young adults with B-ALL who received CAR-T therapy between 2012 and 2019, three cases of t-MN were identified: two AML and one MDS. The median time from CAR-T cell infusion to the diagnosis of t-MN was 1.2 years (range, 0.6–1.6). One patient, who developed AML, had a germline TP53 mutation.[64]

Despite vector integration into sensible genomic sites may theoretically increase the risk of oncogenic potential in engineered cells, no case has yet been reported[67,68]; nevertheless, long-term follow-up remains limited.

In conclusion, the onset of t-MN appears to have a multifactorial origin, mainly related to previous therapeutic lines or pre-existing cytogenetic alterations.

6 | IMMUNE RECONSTITUTION

Patients receiving lymphodepleting chemotherapy followed by CD19 CAR T-cell therapy are at risk of acute and chronic immunosuppression.[1,69] A global overview of immune reconstitution after CAR-T according to current literature is shown in Table 3.

6.1 | Cellular immune reconstitution

Baird et al investigated hematologic recovery, immune reconstitution, and infectious complications in 41 patients with DLBCL treated with axi-cel following fludarabine-cyclophosphamide lymphodepletion.[70] At 1 year after CAR-T infusion, 60% of patients had still a CD4+ T-cell count <200 cells per μL. CD8+ T-cells recovered more rapidly, resulting in a CD4/CD8ratio <1.0 in 66.7% of patients by 18 months. The duration of CD4+ T-cell count <200 cells per μL was significantly longer in older patients, but it was not influenced by previous lines of therapy, bridging regimen, the severity of CRS, the use of steroids. A low CD4 count was not associated with clinical response to CAR-T therapy.

Similarly, in a group of 85 patients with DLBCL, CD4+ T cells were low at a median of 220 cells/μL prior to axi-cel and remained persistently low after axi-cel, with a median CD4 count of 155 cells/μL at 1 year.[72]

In a post-hoc analysis of patients with DLBCL treated with axi-cel on the pivotal ZUMA-1 and ZUMA-9 studies, Strati et al. analyzed immune reconstitution in nine patients at 1 year and seven patients at 2 years.[10] CD8+ T-cells and CD56+ natural killer (NK) cells fully recovered in all patients by 1 year, while reconstitution of CD4+ T cells was delayed, with normalization in only 67% of patients at 1 year. Similarly, in 39 CAR-T recipients with R/R, the absolute counts of CD4+, CD8+, and CD16/56+ lymphocytes decreased significantly after lymphodepleting chemotherapy: while CD8+ and CD16/CD56+ cell counts rapidly recovered in all patients at a median time of 21 days,[7–10,12–15,17,21,22,24–64,66–92] a slower recovery was observed for CD4+, which recovered within 60 days in only 5 (23.81%) of patients.[71]

6.2 | B-cell aplasia

CD19 is expressed on the surface of normal B cells during all maturation stages, from pro-B cells in bone marrow to memory B-cells and

TABLE 3 Incidence of T-cell and humoral toxicities according to major reports.

Reference	Patients	Target	Costimulation	Setting	Early (<day 30)			Prolonged (day 30–90)			Late (>day 90)			Over 1 year		
					CD4	B-cell	IG	CD4	B-cell	IG	CD4	B-cell	IG	CD4	B-cell	IG
Baird[70]	41	CD19	CD28	RL						44%	54%	67%		60%	50%	
Strati[10]	31	CD19	CD28	CT										29%		
Wang[71]	39	CD19	4-1BB	CT				76%	60%							
Logue[72]	85	CD19	CD28	RL		88%			77%			54%			42%	53%
Dejà-Martinez[73]	34	CD19	4-1BB	CT					75%			60%			60%	
Maude[74]	30	CD19	4-1BB	CT					73%							
Hill[37]	133	CD19	4-1BB	CT		98%	35%		79%	27%			46%			
Locke[66]	35	CD19	CD28	CT					83%			39%			25%	

Note: The table indicates the affected blood compartment (e.g., IG for hypogammaglobulinemia). Decreased CD4 count is intended as CD4 lower than 200/μL. B-cell aplasia is assessed according to different authors, as described in the text. Hypogammaglobulinemia is normally intended as levels lower than 400 mg/dL.

Abbreviations: CT, clinical trial; IG, immunoglobulins; Pts, patients; RL, real life.

plasmablasts; due to CAR-T cell expansion and persistence, prolonged B cell aplasia is an expected "on-target off-tumor" effect and it may persist as a long-term issue.

The definition of B-cell aplasia differs between various authors, ranging from <1% CD19+ or CD19/CD20+ cells of the peripheral blood mononuclear cells,[69,71] to CD19+ count less than 3% of all peripheral blood lymphocytes,[74] to less than 0.01% $CD19^+$ normal B cells among blood leukocytes.[37]

B-cell aplasia is reported in up to 98% of patients within 2 weeks to 1 month after CAR-T infusion and may persist for over 6 months, extending to years, as reported in Table 3.

In 85 patients treated with axi-cel for R/R DLBCL, Logue et al reported that B-cells at baseline were present in 48.3% of cases, but only 11.8% of patients had detectable CD19+ B-cells at day 30. After infusion, the proportion of patients with detectable B-cell levels at day 90, 180, and 360 days raised to 22.6%, 46.2%, and 57.9%, respectively.

Other authors reported evidence of endogenous B-cell recovery by day 90 in 21% of patients, with median recovery at day 69.[37] In the ZUMA-1 study, 61% of patients had detectable B cells at 9 months and 75% had detectable B cells at 24 months.[66]

Bhoj et al reported a slower recovery, with persisting B-cell aplasia for a mean of 571 days following CAR-T cell therapy,[69] similar to 60% of patients reported after 12 months from infusion by other authors.[70]

Assessment of peripheral B cells might serve as an effective proxy for ongoing CAR19 activity in the initial months following infusion. B-cells recover with different kinetics when compared to CD4 and CD16/56+. This suggests that B cell depletion after CAR-T cell infusion is caused not only by lymphodepleting chemotherapy but also by the targeted killing function of anti-CD19 CAR-T cells.[71] The CD19+ cell counts negatively correlated with the peripheral blood CD19 CAR-T DNA copies. In 30 patients with B-ALL, the ongoing B-cell aplasia was superior to flow cytometry and as sensible as quantitative PCR in revealing the persistence of CAR-T cells 1 year after infusion. In this study, B-cell aplasia occurred in all the patients who had a response and the probability of relapse-free B-cell aplasia at 6 months was 73% (95% CI 57–94).[74] A longer duration of B-cell aplasia was associated with disease remission status after axi-cel (hazard ratio, 4.66; 95% CI, 1.76–12.29).[70] Interestingly, loss of B-cell aplasia preceded or was concurrent with clinical or radiographic disease relapse or progression in 61.9% of patients (median, 33 days prior; range 0–147), independently from CD19 expression status on lymphoma biopsy at relapse.

In patients with B-cell recovery, Dejà-Martinez described some degree of B-cell dysfunction, documenting low IgM and/or IgA serum levels as well as a defective response to S.typhi vaccination, indicating that functional B-cell reconstitution might take longer than expected from flow cytometry studies. Recovering B-cells were mainly naïve.[73]

6.3 | Hypogammaglobulinemia

Secondary to CD19+ B cell destruction, patients may develop a progressive decrease in all immunoglobulin isotypes (IgG, IgA, and IgM),

in the form of dysgammaglobulinemia (deficiency of one or more, but not all, classes of immunoglobulins) or full agammaglobulinemia.

Hypogammaglobulinemia may be defined as the presence of serum immunoglobulin concentration lower than LLN or IgG < 400 mg/dL. Reported rates of hypogammaglobulinemia may vary widely across studies (Table 3), and most data also do not account for the rate of hypogammaglobulinemia that preceded CAR-T therapy.

According to Logue et al, 27.6% of patients had IgG <400 mg/dL at baseline. IgG nadir was reached 6 months after infusion. Excluding patients censored for receiving IgG, 52.9% had IgG levels >400 mg/dL at 1 year. Also Baird et al demonstrated that serum IgG levels decreased over the first 9 months following axi-cel infusion, with 62% of patients reached a serum IgG level < 400 mg/dL over the course of follow-up, and did not recover within 18 months in 44.4% of patients. Among patients with normal serum IgG levels at baseline, new hypogammaglobulinemia occurred in 29.4% of patients at a median of 2 months post-infusion.

The nadir of IgG was reached in median at day 44 (range 7–153) and recovery occurred in 28.5% of patients at a median of 184 days.[73]

In the cohort of pediatric pts with B-ALL, Deyà-Martinez et al., observed that 71% of patients developed undetectable IgM levels at a median of 71 days after infusion[9,11,14,16–22,36,50–64,66–98]; IgA levels decreased in 13% of patients to undetectable levels at 185 days after infusion (11–308). Patients with undetectable IgM, showed a longer CAR-T persistence (373.5 vs. 60 days; *p*: <.001).

7 | MANAGEMENT OF CYTOPENIAS AND HYPOGAMMAGLOBULINEMIA

In some cases, a cautious wait-and-watch approach to cytopenias may be adequate; when intervention is required, physicians may decide to support patients with transfusions or growth factors.[5,75] Rarely, a hematopoietic stem cell boost may be affordable when some conditions are present. In this section, we detail the main experiences and current approaches.

7.1 | Transfusions

Patients treated with CAR-T may require transfusion support. The proportion of patients receiving red-blood cell and platelet transfusions has been reported to be 63.4% and 34.1%, respectively. More than 30% of transfusions were infused beyond day 28.[70]

The recently published guidelines on the use of irradiated blood components by the British Committee for Standards in Haematology (BCSH) recommended the use of irradiated cellular blood components from 7 days prior to lymphocyte collection until at least 3 months after CAR-T cell infusion.[76]

7.2 | Thrombopoietin receptor agonists (TPO-RAs)

Bone marrow biopsies from patients who had significant and severe prolonged hematologic toxicity after CAR-T therapy were hypoplastic, similar to those of patients with acquired aplastic anemia (AA).[77] The thrombopoietin receptor agonists (TPO-Ras) are used in acquired AA as well as in the management of poor graft failure after allogeneic stem cell transplantation[78,79] and stimulate not only the restoration of thrombopoiesis but also of trilineage hematopoiesis. Some similarities in the pathogenesis of thrombocytopenia post-CAR-T infusion could form the rationale for the use of TPO-RAs for this clinical condition.

In a recent survey on behalf of EBMT and EHA, physicians from 18 centers around the world declared to treat long-lasting thrombocytopenia with TPO-RA in 86% of cases, while transfusion of autologous stem cells and pulse dose steroids appeared to be secondary rescue options.[80] Despite this apparently widespread practice, there are only few reports to support the use of TPO-RAs.[3,81] Nagle et al reported 4 out of 18 (22%) cytopenic patients receiving eltrombopag at doses between 50 and 150 mg daily, with hematological recovery with a median time of 123 days.[77] Six more patients with severe persistent cytopenia treated with eltrombopag and/or romiplostim were reported by Beyar-Katz and colleagues: the median starting time was 43 days after CAR-T infusion. After 1 month all patients obtained complete resolution.[82] In a recent paper, Jain and colleagues suggested starting eltrombopag at a dose of 75 mg daily, with a possible increase to 150 mg daily. Responses were observed in 1–2 months.[5] Romiplostim at the dose of 10 mg/kg weekly may be an alternative treatment.[7]

7.3 | Erythropoietin

Erythropoietin (EPO) in association with irradiated red-blood-cells transfusion is commonly used in the management of prolonged anemia post-CAR-T infusion.[22]

Wang et al reported the experience of 17 cytopenic patients after CART infusion. They were treated with blood transfusion, G-CSF, erythropoietin, and thrombopoietin receptor agonists. However, only two patients had complete hematologic recovery. Patients were treated with low doses of prednisone obtaining complete or partial response with transfusion independency. This seems to support the hypothesis that the underlying mechanism is essentially immunologically mediated.[22]

7.4 | Granulocyte colony-stimulating factor (G-SCF)

The use of G-CSF was initially discouraged in experimental and routine practice due to the similarity between G-CSF and GM-CSF, which is involved in the cytokine storm, and the possible pro-inflammatory role of G-CSF. Some initial data did report increased severity of CRS in patients treated with G-CSF.[83] GM-CSF inhibition reduced CAR-T neurotoxicity in murine models.[84]

Some experiences with prophylactic pegylated or daily G-CSF administration starting 2 days before to 2/5 days after CAR-T infusion have been reported. In those experiences, prophylactic G-CSF

ameliorated the outcomes in terms of incidence of febrile neutropenia (57% vs. 80%), time to ANC recovery (3 vs. 5 days), and hospitalization (17.5 vs. 20 days), while it did not reduce delayed and recurrent severe,[21,87,89–91] the rate of use of antibiotics nor the risk of infections according to several real-life retrospective reports.[14,83,85–87]

In one study, but not in others, prophylactic G-CSF was associated with a superior incidence of grade ≥ 2 (but not grade ≥ 3) CRS,[85] while Barreto and colleagues showed a longer duration of CRS in patients receiving G-CSF, with no difference in CRS incidence.[87] None of those studies reported an impact of G-CSF on ICANS. Some retrospective reports have described worse disease outcomes for patients receiving G-CSF, but it was not confirmed in larger experiences.[86,88] Even though this correlation has not always been observed nor a precise biological rationale has been identified, it cannot be excluded that G-CSF may have some modulating effect on myeloid suppressor cells or on the CD8+ compartment.[89]

Overall, G-CSF administration may reduce the duration of severe neutropenia and seems safe in terms of CRS and ICANS. Currently, EBMT/JACIE/EHA 2022 guidelines suggest administering G-CSF after day 14 in case of persistent severe neutropenia, despite earlier administration may be considered in patients at high risk of infections.[75] In case of concomitant inflammatory CAR-T toxicities, it is cautious to avoid G-CSF. Prospective clinical trials on optimal G-CSF strategies are still required.[5]

7.5 | Hematopoietic stem cell boost

Infusion of autologous stem cells or full allogeneic hematopoietic stem cell transplants (HSCT) has been used to treat persistent and prolonged aplasia.

Gödel and colleagues reported for the first time on the use of stored autologous peripheral blood stem cells (PBSC) for rescuing persistent cytopenia.[90] PBCS were infused at a median of 52 days after CAR-T cells and resulted in a fast hematological recovery.[90] Subsequently, other centers began to rescue cytopenic patients with autologous CD34+ cells. A European survey by Rejeski and colleagues report 13 patients with severe pancytopenia or long-lasting G-CSF support or transfusion dependency receiving autologous CD34+ cell infusion after median of 69 days from CAR-T.[80] In that case, 8/13 patients received unmanipulated CD34+ products. Engraftment was obtained in 82% of cases. More recently, a German report described 31 patients receiving CD34 boosts at a median of 43 days after CAR-T infusion for severe/persisting neutropenia or infections, with a fast (median 9 days) neutrophil recovery in 84% of patients.[91] Notably, the majority (84%) of those infusions were administered in the absence of active infection, while 4 out of 5 patients who received CD34 because of active infection died due to sepsis.

If CAR-T cells were administered after a previous allogeneic HSCT, donor-derived CD34+ boost in patients experiencing persistent grade ≥ 3 cytopenias has also been reported, with no GVHD occurrence.[21,91,92] When sequential treatment with tandem infusion of CAR-T and autologous or allogeneic HSCT was performed inside clinical trials, it was observed that CAR-T did not impact hematopoietic recovery or GVHD after transplants.[93,94]

In a real-life survey, autologous stem cell boosts, when available, were the preferred option for the rescue of persistent neutropenia in 63% of centers, while it was considered a therapeutic option for persisting thrombocytopenia refractory to TPO-RA by 43% of experts. In the same survey, most experts declared they would consider day 90 as a decisional timepoint for considering autologous stem cell boost both for neutropenia or thrombocytopenia. Hematopoietic stem cells were commonly infused with no conditioning regimen in the majority of cases (76%). Routine prophylactic hematopoietic stem cell collection was uncommon.[80]

7.6 | Management of hypogammaglobulinemia

Screening for serum IgG should be routinely performed within the first 3 months after CAR-T infusion. Recommendations and data in the literature on the efficacy of immunoglobulin replacement therapy in patients undergoing CAR-T with hypogammaglobulinemia (IgG < 400 mg/dL or 400–600 mg/dL) and severe or recurrent/chronic infections are limited. In total 27.1% of patients received intravenous immunoglobulins after axi-cel, a proportion similar to the 31% in the ZUMA-1 and 30% in JULIET trials. Immunoglobulin replacement should be used routinely in children and may be considered in adults with hypogammaglobulinemia with or without serious/recurrent infections.[75,95,96] Intravenous or subcutaneous immunoglobulin replacement aims to maintain serum IgG levels >400 mg/dL in adults. Discontinuation of immunoglobulin replacement must be guided by recovery of functional B cells.[36,96,97]

7.7 | Infections and prophylaxis

Opportunistic infections are common in CAR-T recipients, and may occur in 45%–72% of patients, with severe infection in 12%– 48%.[36,97] Active infections should be fully treated and under control prior to the administration of lymphodepletion.

Most infections in the early period after CAR-T infusion, within 30 days, are due to bacteria or respiratory viruses, while invasive fungal infections are rare; differently, viral infections predominate after day +30.[75]

Before vaccines were available, mortality due to SARS-CoV-2 was 41% in CAR-T patients.[98] The adherence to national mRNA-based vaccination programs is recommended, and some data on the efficacy of prophylaxis with the monoclonal antibody tixagevimab/cilgavimab to prevent the onset of symptomatic SARS-CoV-2 infection is available.[36] This clinical behavior may need to be re-assessed in the context of new variants.

Consensus exists on infectious prophylaxis for both viral infections and Pneumocystis carinii pneumonia during early cytopenias, whereas there are no mandatory indications for antibacterial or antifungal prophylaxis in patients with late cytopenias.[75]

Anti-pneumocystis prophylaxis with trimethoprim-sulfamethoxazole or alternative agents for patients with a sulfa allergy (e.g., aerosolized pentamidine, dapsone, or atovaquone orally) is recommended until 1 year from CAR-T infusion and CD4+ T cells > 200/μL. According to current recommendations, systemic antifungal prophylaxis is not routinely recommended in all patients, but the use of posaconazole, fluconazole, or micafungin should be considered in higher-risk patients with a history of fungal infection, severe or prolonged neutropenia, long-term steroid use, or in patients after allogeneic HCT.[5,36,75,97]

8 | CONCLUSIONS

Cytopenia and immune dysregulation after treatment with CAR-T have an early onset and may persist for long-lasting periods.

The overlap between neutropenia, decreased CD4+ count (which may persist in 60% of patients at 1 year after CAR-T infusion) and concomitant B-cell aplasia and hypogammaglobulinemia confer a high infectious risk in CAR-T recipients.

Identification of risk factors and tailoring of prophylactic, preemptive and supportive approaches on all aspects of hematopoiesis are mandatory for the optimal management of this common and challenging toxicity of CAR-T programs.

AUTHOR CONTRIBUTIONS

Eugenio Galli and Gina Zini, conceived and designed the manuscript. Eugenio Galli, Ilaria Pansini, Federica Sorà, Francesco Autore, Filippo Frioni, Elisabetta Metafuni, Maria Assunta Limongiello, Sabrina Giammarco, Alberto Fresa, Idanna Innocenti, Elena Maiolo, and Silvia Bellesi wrote the paper. Federica Sorà, Gina Zini, and Patrizia Chiusolo supervised the review and revised the paper. All authors have critically revised and approved the paper.

ACKNOWLEDGMENTS

The authors acknowledge the support of "Centro di Ricerca sulle cellule staminali emopoietiche e le terapie cellulari, Università Cattolica del Sacro Cuore, Roma" research funding. The authors express their gratitude to Professor Simona Sica, Professor Stefan Hohaus, Professor Andrea Bacigalupo, and Professor Luca Laurenti, for providing their invaluable guidance, comments, and suggestions throughout the course of the project.

FUNDING INFORMATION

The authors received no funding for the present research paper.

CONFLICT OF INTEREST STATEMENT

EG received travel grants from Kite Gilead and honoraria for speaker's bureau from Novartis. PC received honoraria for participating to advisory boards from Bristol Myers Squibb and Novartis. All other authors declare no competing conflict of interest.

DATA AVAILABILITY STATEMENT

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How to cite this article: Galli E, Fresa A, Bellesi S, et al. Hematopoiesis and immune reconstitution after CD19 directed chimeric antigen receptor T-cells (CAR-T): A comprehensive review on incidence, risk factors and current management. *Eur J Haematol*. 2024;112(2):184-196. doi:10.1111/ejh.14052