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nonacog beta pegol

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This Novo Nordisk advertisement is intended for UK Healthcare Professionals

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Refixia® ONCE-WEEKLY PROPHYLAXIS,[1] GIVING YOUR PATIENTS THE CONFIDENCE TO LIVE BEYOND HAEMOPHILIA B[2-4]

With Refixia® prophylaxis, adolescents (12 years and above) and adults spent approximately 80% of the week with FIX activity levels in the non-haemophilia range (FIX activity higher than 40%)*[2]

EHL Comparison Video
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Leopoldo, 61 years old, is an IT engineer and loves spending time sailing. Leopoldo lives with haemophilia B.

Prescribing Information

Refixia®▼ Refixia® 500 IU Refixia® 1000 IU Refixia® 2000 IU (Powder and solvent for solution for injection) Nonacog beta pegol. Nonacog beta pegol is recombinant human factor IX, produced in Chinese Hamster Ovary (CHO) cells by recombinant DNA technology, covalently conjugated to a 40 kDa polyethylene-glycol (PEG). Refixia® contains approximately 125 IU/ml, 250 IU/ml and 500 IU/ml after reconstitution. **Indication:** Treatment and prophylaxis of bleeding in patients 12 years and above with haemophilia B (congenital factor IX deficiency). **Posology and administration:** Prophylaxis: 40 IU/kg body weight once weekly. Adjustments of doses and administration intervals may be considered based on achieved FIX levels and individual bleeding tendency. Patients on prophylaxis who forget a dose are advised to take their dose upon discovery and thereafter continue with the usual once weekly dosing schedule. A double dose should be avoided. On demand treatment: Dose and duration of the substitution therapy depend on the location and severity of the bleeding. Early haemarthrosis, muscle bleeding or oral bleeding / more extensive haemarthrosis, muscle bleeding or haematoma: recommended dose of 40 IU/kg of Refixia - single dose is recommended to treat bleeding. Severe or life threatening haemorrhages: recommended dose of 80 IU/kg of Refixia - additional doses of 40 IU/kg can be given. Surgery: Minor surgery including tooth extraction: recommended dose of 40 IU/kg body weight - additional doses can be given if needed. Major surgery: 1) recommended dose of 80 IU/kg body weight - pre-operative dose. 2) recommended dose of 40 IU/kg body weight - consider two repeated doses of 40 IU/kg (in 1–3 day intervals) within the first week after surgery. Due to the long half-life of Refixia, the frequency of dosing in the post-surgical period may be extended to once weekly after the first week until bleeding stops and healing is achieved. *Intravenous use.* Intravenous bolus injection over several minutes after reconstitution of the powder for injection with the histidine solvent. The rate of administration should be determined by the patient's comfort level up to a maximum injection rate of 4 ml/min. **Contraindications:** Hypersensitivity to the active substance, or to any of the excipients, or to hamster protein. **Special warnings and precautions for use:** Hypersensitivity Allergic type hypersensitivity reactions are possible with Refixia. The product contains traces of hamster proteins. If symptoms of hypersensitivity occur, patients should be advised to discontinue use of the medicinal product immediately and contact their physician. Patients should be informed of the early signs of hypersensitivity reactions including hives, generalised urticaria, tightness of the chest, wheezing, hypotension, and anaphylaxis. In case of shock, standard medical treatment for shock should be implemented. Inhibitors After repeated treatment with human coagulation factor IX (rDNA) products, patients should be monitored for the development of neutralising antibodies (inhibitors) that should be quantified in Bethesda Units (BU) using appropriate biological testing. There have been reports in the literature showing a correlation between the occurrence of a factor IX inhibitor and allergic reactions. Therefore, patients experiencing allergic reactions should be evaluated for the presence of an inhibitor. It should be noted that patients with factor IX inhibitors may be at an increased risk of anaphylaxis with subsequent challenge with factor IX. Because of the risk of allergic reactions with factor IX products, the initial administrations of factor IX should, according to the treating physician's judgement, be performed under medical observation where proper medical care for allergic reactions could be provided. In case of residual FIX activity levels, there is a risk of interference when performing the Nijmegen modified Bethesda assay for inhibitor testing. Therefore a pre-heating step or a wash-out is recommended in order to ensure detection of low-titre inhibitors. Thromboembolism: Because of the potential risk of thrombotic complications, clinical surveillance for early signs of thrombotic and consumptive coagulopathy should be initiated with appropriate biological testing when administering this product to patients with liver disease, to patients post-operatively, to new-born infants, or to patients at risk of thrombotic phenomena or DIC. In each of these situations, the benefit of treatment with Refixia should be weighed against the risk of these complications. Cardiovascular event In patients with existing cardiovascular risk factors, substitution therapy with FIX may increase the cardiovascular risk. Catheter-related complications If a central venous access device (CVAD) is required, risk of CVAD-related complications including local infections, bacteraemia and catheter site thrombosis should be considered. Paediatric population Refixia is not indicated for use in children (below 12 years). The listed warnings and precautions apply both to adults and adolescents (12–18 years). Sodium content This medicinal product contains less than 1 mmol sodium (23 mg) per vial, i.e. it is essentially "sodium-free". **Fertility, pregnancy and lactation:** Animal reproduction studies have not been conducted with factor IX. Based on the rare occurrence of haemophilia B in women, experience regarding the use of factor IX during pregnancy and breastfeeding is not available. Therefore, factor IX should be used during pregnancy and lactation only if clearly indicated. **Undesirable effects:** Common (≥ 1/100 to < 1/10): nausea, pruritus (terms pruritus and ear pruritus), fatigue, injection site reactions (injection site pain, infusion site pain, injection site swelling, injection site erythema and injection site rash). Uncommon (≥ 1/1,000 to < 1/100): hypersensitivity, palpitations, hot flush. Unknown (cannot be estimated from the available data): anaphylaxis, inhibitors. Hypersensitivity or allergic reactions have been observed rarely with recombinant factor IX products and may progress to severe anaphylaxis (including shock). In some cases, these reactions have progressed to severe anaphylaxis, and they have occurred in close temporal association with development of factor IX inhibitors. Nephrotic syndrome has been reported following attempted immune tolerance induction in haemophilia B patients with factor IX inhibitors and a history of allergic reaction. The Summary of Product Characteristics should be consulted for a full list of adverse reactions.

MA numbers and Basic NHS Price:

Refixia® 500 IU	EU/1/17/1193/001	£1,221.50
Refixia® 1000 IU	EU/1/17/1193/002	£2,443.00
Refixia® 2000 IU	EU/1/17/1193/003	£4,886.00

Legal category: POM.

For full prescribing information, please refer to the Summary of Product Characteristics which is available: Novo Nordisk Limited, 3 City Place, Beehive Ring Road, Gatwick, West Sussex, RH6 0PA. **Marketing Authorisation Holder:** Novo Nordisk A/S, Novo Allé, DK-2880 Bagsværd, Denmark. **Date last revised:** July 2018.

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store. Adverse events should also be reported to Novo Nordisk Limited (Telephone Novo Nordisk Customer Care Centre 0845 6005055).
Calls may be monitored for training purposes.

* Adolescents and adults with haemophilia B treated once weekly with Refixia® 40 IU/kg are predicted to have a FIX activity higher than 40% for 130 hours out of 168 hours, equal to approximately 80% of the week.[2]

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1. Refixia® Summary of Product Characteristics.
2. Tiede A et al. Haemophilia 2017;23(4):547-555.
3. Negrier C et al. Haemophilia 2016; 22 (4): 507-513.
4. Collins PW et al. Blood 2014; 214 (26): 3880-3886

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CAR-T cell therapy in paediatric acute lymphoblastic leukaemia – past, present and future

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Summary

Over the last decade, chimeric antigen receptor (CAR)-T cell therapy has emerged as a promising treatment modality for relapsed/refractory B-cell malignancies in both children and adults. As an adoptive immune therapy, CAR-T cells have the potential to overcome disease that is resistant to chemo- and radiotherapy as well as represent a viable option for those who have already reached toxicity ceilings with standard therapies. CD19-directed CAR-T cell products have been licensed for use in paediatric B-cell acute lymphoblastic leukaemia that is refractory, in relapse post-transplant or in second or later relapse. Many challenges remain, rightly resulting in a heavily-mined research field. These include mitigating short-term immune-mediated toxicity, maintaining durability of responses, broadening treatment accessibility and extending its applicability to other malignant settings. In this review, dedicated to marking 60 years since the establishment of the British Society for Haematology, we will focus on the contribution of our community towards the success of CD19-directed CAR-T cell therapy in children. We will put current practice in CAR-T cell therapy into the context of future challenges to be addressed in order for it to fulfil its "game-changing" therapeutic potential.

Keywords: chimeric antigen receptor T cell, acute lymphoblastic leukaemia, paediatric, adoptive T-cell therapy.

The modern concept of T cell immunotherapy started with the first bone marrow transplants and recognition of the graft-versus-leukaemia effect, which was established on the basis of seminal observations made between 1979 and 1990. In the UK, John Goldman and colleagues established a role for allogeneic stem cell transplantation (SCT) in chronic myeloid leukaemia, where they observed a higher rate of relapse in patients receiving T cell depleted bone marrow[1] and later successfully demonstrated that adoptive cell therapy with donor lymphocyte infusions (DLI) could be used for subsequent relapses.[2–4] Further, transfer of a functional immune system into an immunocompromised host resulted in eradication of haematopoietic precursors and severe bone marrow aplasia as a result of transfusion-associated graft-versus-host disease (GVHD).[5] Finally, patients with acute or chronic GVHD had a lower rate of leukaemic relapse than those without this complication, and a higher incidence of relapse was noted in transplants from a syngeneic twin.[6,7] Further contributions to this field have been achieved by recognising the value of virus-specific cytotoxic lymphocytes[8–12] in treating viral infections following SCT. Discovery of tumour infiltrating lymphocytes (TIL), which could be expanded *ex vivo* and used to induce remission, (e.g. in melanoma patients) reinforced the view that T cells can mediate cancer cures, but these could not be not isolated from all patients.[13,14] Administration of antibodies mediating checkpoint blockade can release the endogenous T cell repertoire from inhibition, uncovering anti-tumour responses in a variety of cancers in which the burden of neo-antigens is high[15] and eliminating the need to isolate T cells from individuals. Furthermore, since the specificity of a T cell can be re-directed solely through expression of a novel T-cell receptor (TCR), a strategy of viral transduction of lymphocytes with high-affinity TCRs specific for tumour-associated antigens was introduced.[16] Emma Morris and Hans Stauss were among the first in the UK and in Europe to undertake a clinical study of Wilms' tumour (WT1) TCR-transduced T cells,[17] an approach which broadens the applicability of the adoptive transfer of anti-tumour T cells. However, TCR gene-engineering approaches are limited to patients with particular human leucocyte antigen (HLA) backgrounds. Therefore, to be relevant to a broader range of patients, interest grew in generating synthetic receptors recognising antigens in an unrestricted way.

Constructing chimeric antigen receptors

Chimeric antigen receptors offered such an appeal by combining an antigen recognition domain usually derived from an antibody (e.g. a single-chain variable fragment or scFv), with a hinge/stalk region, a transmembrane domain tethering the receptor to the cell membrane and connected

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intracellularly to the CD3ζ endodomain in a single linear molecule (Fig 1). Antibodies can be more flexibly-derived[18] against a range of cell surface antigens and are not restricted to peptide recognition, but can be raised against lipid/carbohydrate and other moieties. Such first generation CARs recognise tumour cells and achieve target cell lysis.[19] However, they fail to achieve full T-cell activation and, crucially, lack robust cytokine production and proliferation that are needed for T-cell expansion and persistence.[20,21] Second generation CARs incorporate compound endodomains, including domains from co-stimulatory molecules (such as CD28, 4-1BB and OX40), as well as signalling domains from CD3ζ.[22–24] Such CARs demonstrate improved cytokine secretion and higher proliferative responses.[25–28] Because of their flexible and modular design, CARs can be engineered with a range of domains derived from different signalling molecules to modify T-cell effectiveness. Third generation CARs typically include a CD28 domain followed by additional co-stimulatory endodomains (such as OX-40 or 4-1BB).[29,30] CAR-T cell potency can be further enhanced through inclusion of additional transgenes, for example, encoding cytokines (e.g. IL-12), additional co-stimulatory ligands or other secreted mediators (e.g. scFvs to block co-inhibitory receptors).[31,32] These are termed "armoured" fourth generation CAR-T cells (Fig 1).

CD19 CAR-T cell therapy

CAR-T cells showed promising results against CD19-positive primary B-cell acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL) cells *in vitro*.[33–35] This was followed by promising *in vivo* models of CD19 CAR-T cell therapy of human B-cell malignancies.[34,36–38] However, immunodeficient murine models were inadequate to investigate CAR-T cell persistence, and the first clinical studies with first generation CD19 CAR-T cells used in refractory follicular lymphoma demonstrated a lack of persistence.[39] Subsequent studies using second generation CAR-T cells suggested that this design results in more sustained expansion/persistence of CAR-T cells, irrefutably demonstrated when Savoldo et al. co-administered first and second generation CAR-T cells and noted clear superiority in expansion and proliferation of the latter population.[37,40]

The doubling time of aggressive ALL was felt to be too rapid for CAR-T cell therapy to be successful, yet the first two paediatric patients with refractory ALL treated on a compassionate basis with second generation CD19 CAR-T cells achieved complete remission (CR), one relapsing with CD19-negative (CD19-) disease 2 months later.[41] Subsequent studies consolidated this information showing CR in 70–96% of patients with advanced disease, with attainment of a measurable residual disease (MRD) negative status in 60–93% of patients after CAR-T cell treatment.[42–45]

Current practice

Following a successful single centre study at the University of Pennsylvania,[42] the multicentre ELIANA study demonstrated the efficacy of tisagenlecleucel (Kymriah, Novartis), a CD19 CAR-T cell product incorporating the FMC63 CD19 binder in a second generation format with a 4-1BB costimulatory domain in paediatric relapsed or refractory ALL.[45] Overall survival and event-free survival at 12 months were 81% and 50% respectively. This pivotal study led to licencing by the Food and Drug Administration (FDA) in the United States and European Medicines Agency (EMA) for treatment of children and young adults with relapsed and refractory ALL. The main adverse events reported included cytokine release syndrome (CRS), neurotoxicity, cytopenias and B-cell aplasia leading to hypogammaglobulinaemia. Overall, CRS was observed in 77% of patients, with severe (grade 3/4) CRS noted in 46%. Neurotoxicity of any grade was observed in 40% of patients. The median onset of CRS was at 3 days (range 1–51) and median duration of CRS was 8 days (range 1–36). Neurotoxicity appeared at a median onset of 6 days (range 1–359) and had a median duration of 6 days.[46] This promising data, offering hope of a cure for patients relapsing after stem cell transplant, caught the attention of Sir Simon Stevens,[47] who, following fast-tracked NHS England (NHSE) approval, championed a UK delivery framework for tisagenlecleucel (Fig 2).

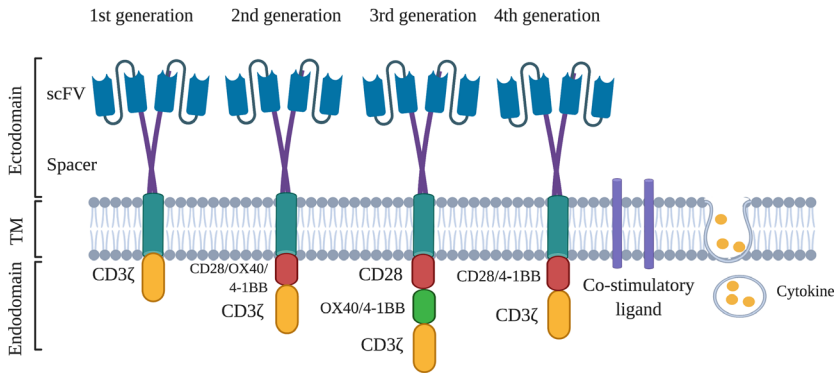

Fig 1. Structure of different generations of chimeric antigen receptors. scFV, single-chain variable fragment.

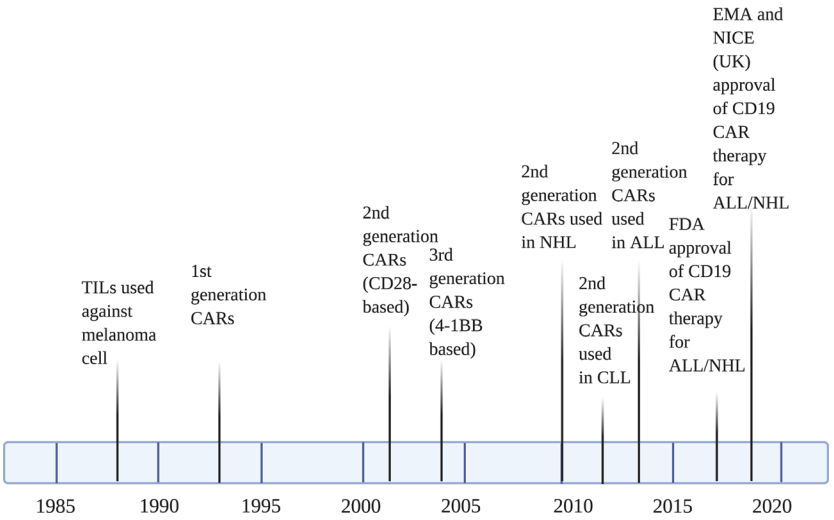

Fig 2. Timeline showing major milestones in development and implementation of CAR-T-cell therapy into clinical practice. ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; EMA, European Medicines Agency; FDA, Food and Drug Administration; NHL, non-Hodgkin lymphoma; NICE, National Institute of Health and Clinical Excellence; TILs, tumour infiltrating lymphocytes.

Referral for NHSE-funded CAR-T cell therapy

National Health Service England (NHSE) established a National CAR-T Clinical Panel for ALL (NCCP ALL) to confirm eligibility for and provide prompt access to tisagenlecleucel. Eligibility criteria are broadly similar to the ELIANA study inclusion criteria.

Eligible patients are allocated to a Joint Accreditation Committee-International Society for Cellular Therapy and EBMT (JACIE)-accredited CAR-T cell therapy centre on the basis of geographic distance, patient preference and local capacity constraints. There are currently 10 centres across the UK that deliver tisagenlecleucel to children and young adults. Funding is provided from the Cancer Drug Fund, with service delivery costs covered by a national tariff.

Tisagenlecleucel manufacture

After patient assessment in the CAR-T cell centre, and once appropriate intervals from last therapy have been observed, peripheral blood mononuclear cells (PBMCs) are collected by leukapheresis and then transferred for manufacture. PBMCs are cultured with T cell mitogenic stimulants and transduced with lentiviral vector to express CARs (Fig 3). This process usually takes between 4–6 weeks.

Bridging chemotherapy

In keeping with the relapsed/refractory nature of the cohort, 87% of patients in the ELIANA study were treated with bridging chemotherapy following leucapheresis.[45] The choice of bridging chemotherapy depends on an assessment of prior toxicity and disease response, the expected interval to admission for CAR-T cell infusion and pre-existing disease burden. Patients with low level disease may be sustained with maintenance-type regimes; for patients with a higher disease burden, escalating vincristine and methotrexate (as in Capizzi interim maintenance but without asparaginase), a 3–4 drug induction, cyclophosphamide/cytarabine or inotuzumab, may be considered.

The principles of bridging chemotherapy are to provide disease control and limit disease burden at the point of CAR-T cell therapy, whilst minimising toxicity. This represents a shift in treatment goals for relapsed/refractory disease compared to prehaematopoietic stem cell transplantation and in front-line therapy, where the reduction of MRD to predefined levels is the goal. The advantage of reducing the disease burden is to reduce the risk of severe CRS;[48] however, this needs to be balanced against provision of CD19 (on disease or normal B cells), to facilitate CAR-T expansion and persistence. Limited data in adult populations indicates that higher intensity bridging therapy is associated with a higher risk of infectious complications without benefit in CAR-T outcomes.[49] Furthermore, shorter persistence of CAR-T cells was noted in patients who had less than 15% $CD19^{+}$ bone marrow cells prior to infusion.[44]

There is some evidence that blinatumumab [a bispecific T-cell engager (BiTE) directed towards CD19], should be avoided as bridging therapy where possible, because of a reduction in CD19 expression levels, which was associated with reduced response rates in a single centre retrospective study.[50]

Lymphodepletion

Lymphodepletion reduces competition for homeostatic cytokines, such as IL-7 and IL-15, deletes regulatory T cells and, potentially, other regulatory immune subsets as well as

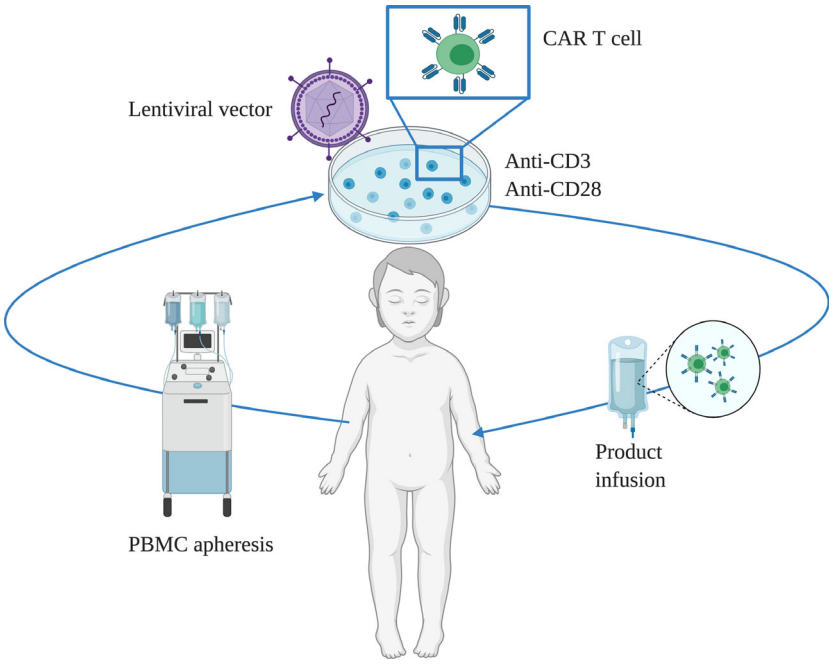

Fig 3. Tisagenlecleucel CAR-T-cell manufacturing process. Peripheral blood mononuclear cells (PBMCs) are collected using leukapheresis and cryopreserved. T cells are thawed and activated using anti-CD3 and anti-CD28 antibodies on the surface of magnetic beads. Stimulated T cells are transduced with a lentiviral vector to express CARs and expanded in a bioreactor for a number of days before the product is cryopreserved, and infused once the product has been assessed to meet a number of regulated quality measures (release criteria).

eliminating anti-CAR immune responses in a proportion of patients. Shorter persistence of CAR-T cells was observed when using single-agent cyclophosphamide compared to the combination of cyclophosphamide and fludarabine.[51] This highlights the importance of lymphodepletion intensity, although in some studies, lymphodepletion was optional in the case of leucopaenia.[45] The most common lymphodepletion protocols for paediatric ALL indications include fludarabine and cyclophosphamide.

Complications of CAR-T cell therapy

Cytokine release syndrome

Cytokine release syndrome is a well-recognised complication with the presence of fever in the mildest cases, but proceeding to hypotension, hypoxia, respiratory failure, renal failure, capillary leak and coagulopathy[52] when severe. There have been several iterations of CRS definition, including different grading systems. The American Society for Transplantation and Cellular Therapy provided consensus guidelines in 2018 defining the severity of CRS based on clinical parameters, (i.e., fevers, hypoxia and hypotension). This consensus has been increasingly accepted in the UK and worldwide.[53] The severity of CRS is associated with higher tumour burden,[48] but a clear association with an anti-tumour effect has not been confirmed.[42,43,54–56]

Treatment of CRS depends on its severity and is based on supportive care along with judicious administration of the IL-6 blocking antibody tocilizumab. In the ELIANA study, grade 1 and 2 CRS were reported in 30% of patients, and grade 3 and 4 CRS in 46%.

Rarely, patients with severe CRS do not respond to tocilizumab and require corticosteroids.[48,53,57] However, the use of corticosteroids might dampen T cell function and proliferation.[56,58]

Neurotoxicity

The pathophysiology of neurotoxicity is not fully understood, but is likely to involve impairment of the blood-brain barrier following activation of endothelial cells and the monocytes/macrophage system.[59,60] Neurotoxicity from CAR-T cell therapy, termed immune effector cell-associated neurotoxicity syndrome (ICANS) can manifest as delirium, encephalopathy, aphasia, lethargy, difficulty concentrating, agitation, tremor, seizures and, rarely, cerebral oedema.

The neurotoxicity grading system was revised in 2018, when four grades were defined based on the presence of encephalopathy, depressed level of consciousness, seizures, motor weakness and signs of increased intracranial pressure.[53] However, diagnosing ICANS continues to be a challenge in younger children or those lacking sufficient cognitive ability to be evaluated with existing encephalopathy assessment tools.[53,61]

The severity of neurotoxicity is specific to the CAR-T cell product used. CARs containing CD28 co-stimulatory domain have a higher incidence of neurotoxicity in paediatric and adult populations.[62,63] In the ELIANA study, neurotoxicity was noted in 40% of patients, with grade 3 neurotoxicity noted in 13% (there was no grade 4 neurotoxicity).

The treatment of neurotoxicity depends on the severity of symptoms. In the majority of cases, symptoms resolve over a matter of weeks with supportive care alone. Grade 2 neurotoxicity in the context of CRS should be treated with IL-6 axis blockade with tocilizumab. However, grade 3/4 neurotoxicity unresponsive to tocilizumab or occurring in the absence of CRS, should be treated with corticosteroids.[45,59,64,65]

B cell aplasia

Targeting CD19 with CAR-T cells causes on-target off-tumour depletion of normal B cells such that B cell aplasia is a useful marker of CAR-T cell persistence. Resultant hypogammaglobulinaemia is more likely to develop in children than adults, probably due to lack of a mature (CD19-) plasma cell pool.[66] However, this is easily managed with regular immunoglobulin replacement.[45,64] Current paediatric practice is to replace immunoglobulin with a threshold of 5 g/l. However, prospective data on immunoglobulin replacement after CAR-T cell therapy is lacking.[64,70]

Cytopaenias

Cytopaenias are a well-recognised adverse effect that may persist for several weeks after CAR-T cell therapy. While the exact pathophysiology of cytopaenia is not fully known, it seems important contributing factors are cytokines released during CRS, lymphodepleting regimen, prior chemotherapy or SCT and macrophage activation.[57,67,68]

In the ELIANA study, grade 3 or 4 neutropaenia had not resolved by day 28 in 53% of patients. Similarly, grade 3 or 4 thrombocytopaenia was observed in 41% of patients, beyond day 28. Prolonged neutropaenia may be supported with myeloid growth factors, but they are not recommended in the first 3 weeks following tisagenlecleucel or until CRS has resolved.[46]

Cytopaenias and subsequent infections have been related to the severity of CRS.[69,70] Approximately 20–40% of patients will develop infection within first month following CAR-T cell therapy.[64] The increased incidence of infection in children and young adults seems to decrease after the first 4 months.[71] Severity and incidence of late infections after CAR-T cell therapy are not well described, but most seem to be mild with a predominance of viral respiratory illnesses.[69,72]

The future of CAR-T cell therapy and the contribution of UK-based haematologists

There remain significant challenges to overcome within CD19 CAR-T cell therapy for B-cell malignancies, even though this therapy is now established as a game changer for those with relapsed/refractory ALL. The initial scientific progress was fuelled mainly by US research groups in partnership with pharmaceutical companies, for example, Novartis and Kite, leading to FDA approval. However, the credentials of the UK CAR-T cell research community, discussed below, supported a rapid implementation of a broad range of clinical studies, with the University College London (UCL) portfolio being one of the most advanced and diverse in Europe, as measured by one of the highest patent registrations outside of the US.[73] It is committed to delivering next-generation CAR-T products serving the areas of unmet clinical need.

This expertise was developed through transatlantic collaborations and mentorship of UK-based haematologists, including Dr Martin Pule and Prof Persis Amrolia, by Prof Malcolm Brenner, formerly of the Royal Free and Great Ormond Street Hospitals, but who subsequently established a T cell engineering laboratory in Baylor College of Medicine, Houston, Texas. Since their return to the UK, Dr Pule and Prof Amrolia have implemented one of the first studies of CAR-T cell therapy in Europe (the CD19 TPALL study), and have groups actively researching CAR-T cell therapies for a range of indications as well as developing next-generation CD19 CAR-T cell therapies. Dr Pule founded Autolus Ltd with a broad portfolio of CAR-T cell studies, including for ALL, B-cell non-Hodgkin lymphoma (NHL), myeloma and T cell malignancies. Other CAR-T researchers at UCL include Prof Karl Peggs, Dr Claire Roddie, Prof Waseem Qasim (an immunologist), Prof John Anderson and Dr Karin Straathof (both oncologists) who have translated universal CD19 targeting T cells and GD2 CAR-T cell therapy in neuroblastoma respectively. Graduates of the Sadelain laboratory at Memorial Sloane Kettering Cancer Centre, such as Prof John Maher (an oncologist) and Dr Reuben Benjamin, have established track records in preclinical and clinical studies of CAR-T cells to treat solid organ and haematological malignancies respectively. Prof Katy Rezvani originally trained at the Hammersmith Hospital, and studied under Prof John Barrett at the National Institutes of Health, before establishing a laboratory at the MD Anderson Cancer Centre in Texas, where she has developed a ground-breaking alternative source of CAR effector cells. CD19 CAR natural killer (NK) cells were utilised in a study treating patients with CLL and NHL derived from cord blood donors, and were tested for universal application since they lack an endogenous TCR, and therefore do not mediate GVHD.[74]

Broadly, the greatest challenge is in widening the accessibility of this approach to other cancer settings, including treating solid organ malignancies. But even within therapy of B-ALL, CAR-T cell therapies need to be refined. Relapse and loss of CAR-T cell persistence remain the biggest obstacles, such that at least 50% of patients will need to go on to have further therapy,[64] often involving stem cell therapy. The latter represents a significant burden of toxicity in often heavily

pre-treated patients, as well as a resource burden for healthcare services. However, in some studies, particularly in the setting of a non-persisting CAR-T product, has been associated with improved outcomes compared to patients receiving CAR-T cell therapy without an adjunctive transplant.[44,75,76]

Disease relapse is contributed to by either antigenic escape (usually in the context of persisting CAR-T cells) or loss of CAR-T cell persistence, in which case relapses often continue to express CD19. The relative proportions of $CD19^+$ and $CD19^-$ relapses vary depending on the CAR-T cell product being studied, with a greater rate of $CD19^-$ relapse in patients treated with more persistent CARs.

Antigenic escape

Seven to 25% of all patients treated with CD19-targeting CAR-T cells relapse with $CD19^-$ disease.[77] Therefore, antigenic escape represents an important challenge in the further development of CAR-T cell therapy, not least because such relapses are not amenable to therapy with blinatumomab, a bispecific antibody with efficacy in bridging relapsed patients to stem cell transplant.[78]

Targeting of multiple rather than single antigens with CAR-T cells may theoretically overcome stochastic mechanisms of antigen negative escape because of the low likelihood that two such events would happen at the same time.

There are different strategies in a dual targeting approach for B cell malignancies. Schultz et al. used a bivalent CAR approach targeting CD19/CD22 in a joint paediatric and adult study. In their interim data analysis, 92% of these patients achieved CR at day 28, with only one patient experiencing severe CRS and ICANS. Three patients relapsed with CD19 positive disease due to short product persistence.[79]

Co-administration of separate CD19 and CD22-targeting CAR-T cells is another strategy. In a Chinese study, CR was achieved in 96% of patients. However, again, there was limited persistence of CAR-T cells with nearly 50% of patients relapsing with $CD19^+/CD22^+$ disease.[80] Gardner et al. studied co-administration of three separate populations – single CD19 and CD22 CAR-T cells along with a bispecific CD19/CD22 CAR-T cells. Patients achieved 87% MRD negative CR rates, but observed short persistence of CD22 CARs[81] as a result of which the majority of relapses were with CD22-expressing disease.

In a preliminary report regarding the UK AMELIA study, one of the first clinical studies using a bicistronic CD19/CD22 CAR vector ensuring that all CAR-T cells expressed both CARs, seven out of seven patients (100%), receiving an intermediate or higher cell dose ($>3 \times 10^6$/kg), achieved molecular remission. Three relapses were reported, including one with CD19 negative/CD22 low expression at 1 year after treatment. No severe (grade 3 or 4) CRS was observed.[65] This approach proved to have a favourable toxicity profile, with equivalent response rates, though shorter CAR-T persistence than in the ELIANA study (median 180 days), and $CD19^-$ relapses were still seen, despite the dual targeting strategy.

The shorter CAR-T persistence noted with all these studies compared to tisagenlecleucel, possibly relates to steric hindrance of CD22: CD22 CAR interactions with poorer resultant T-cell activation, which will need addressing in future CAR designs.

Increasing CAR-T cell persistence

Relapse also results from a loss of CAR-T cell persistence. Preclinical studies have shown that generating CAR-T cells with a less differentiated status improves outcomes.[82,83] Around the globe, various groups have investigated different strategies to achieve this, for example, pre-selecting central memory T cells (T_{CM}) or stem cell-like memory T cell (T_{SCM}) populations[51,75,84,85] for CAR transduction, or manufacturing CAR-T cells in the presence of mediators that preserve early differentiation states.[86] CAR-T cells can be further engineered with additional co-stimulatory ligands to improve CAR-T cell efficacy, expansion and persistence, for example, 4-1BBL.[31,32]

Strategies to enhance CAR-T cell signalling/potency and overcome co-inhibitory signalling may, paradoxically, impair CAR-T cell fitness by enhancing T cell exhaustion. CARs generally employ antibody-derived binding domains binding antigens at an affinity range far higher than that of native TCRs, further, selection of particular transmembrane and co-stimulatory domains can lead to T cell exhaustion.[87,88] Thus, more recently, engineering strategies decreasing CAR signalling intensity have shown promise, for example, reducing the immunoreceptor tyrosine-based activation motif (ITAM) number in the CAR signalling domain[89] or altering the promoter used to reduce CAR expression. We translated a novel CD19 binder with a lower affinity for CD19. This CAR was demonstrated to achieve comparable anti-tumour efficacy compared to Kymriah, whilst showing improved expansion (the maximal CAR-T cell concentration achieved was three times higher than that seen with tisagenlecleucel) and persistence, despite treating patients with very low bone marrow disease burden. The median duration of CAR-T persistence was 215 days (range 14–728 days) in 14 evaluable patients. One year overall survival and event-free survival was comparable to the ELIANA study at 63% and 46% respectively.[90] The toxicity profile was very favourable, with no incidence of severe CRS, even when treating patients in frank relapse, and this was confirmed with the use of the same CAR construct in a population of adult patients with advanced ALL,[91] ultimately leading to the launch of a licensing study for this product.[92]

Accessibility

Prior to regulatory approval by the FDA, EMA and the UK National Institute for Health and Clinical Excellence (NICE), patients could only access CAR-T cell therapy on clinical

studies. In the UK, thanks to the pioneering work of haematologists such as Prof Persis Amrolia, Dr Martin Pule, and Prof Paul Veys, along with other UCL investigators, children with relapsed/refractory B-ALL have been able to access a range of open clinical studies at UCL's Institute of Child Health and Great Ormond Street Hospital since 2014. The UK has championed the approach of centralised funding through the NHS for licensed CAR-T cell products and ensured that CAR-T cell therapy is available to everyone eligible.[93] However, there remain many barriers to CAR-T cell therapy even for patients with licensed indications.

A major contributor to global inequity of access to CAR-T cell therapy is its price, which certainly precludes wide access in a developing world setting. The significant costs result from production on a per-patient basis in centralised manufacturing facilities delivering products at clinical grade and meeting strict quality criteria.[94] There are also the high costs of key reagents such as lentiviral transfer vectors. There have been advances in the CAR-T cell manufacturing process, including non-viral transduction and use of semi-automated platforms, such as developed by Dr Claire Roddie and Prof Mark Lowdell at UCL, which provide de-centralised/point of care production,[95,96] however, these advances have not yet progressed to a licensed product.

Apart from limited healthcare resources, access to CAR-T cell therapy may be prevented because of factors such as patient lymphopenia precluding autologous harvest, manufacturing failure, rapid disease progression and patient co-morbidity. As a result, a significant number of patients screened for eligibility failed to be infused on studies such as ELIANA (32 of 107 screened) and CARPALL (three of 17 patients screened).[45,90] Such patients would benefit from access to an off-the-shelf, universal CAR product without the inherent 5–8 weeks delay of autologous manufacture and associated bridging therapy. Global research efforts have yielded a number of avenues for delivery of universally-applicable CAR products, including providing CAR transduced effector immune cells without a propensity for yielding immune responses against an allogeneic recipient (e.g. NK cells)[74] without the need for gene-engineering to achieve universal application. At UCL, Pule et al. developed a concept of transcription activator-like effector nuclease (TALEN) TCR gene-disrupted T cells, rendered also resistant to alemtuzumab through concomitant CD52 disruption, in collaboration with the biopharmaceutical company Cellectis. As such, these CAR-T cells lacking an endogenous TCR would not be capable of mediating anti-host responses and would not in turn be deleted from the host through application of alemtuzumab as a conditioning agent. After promising pre-clinical work and good manufacturing practice procedures, a successful pilot study in two infants with refractory ALL at Great Ormond Street Hospital led to a multicentre worldwide clinical study.[97] There is a potential for genotoxicity with transcription activator-like effector nucleases (TALEN)-mediated gene disruption, which has led to consideration of safer, next-generation gene-editing platforms, such as base-editing[98] as well as innovative protein engineering approaches.[99]

Novel targets, novel constructs and beyond

There are ongoing clinical trials assessing the impact of CD19 CAR-T cell therapy in paediatric patients with relapsed/refractory B cell NHL.[100,101] However, there is an even larger unmet clinical need for finding treatment options for non-B cell haematological malignancies. Relapsed/refractory T-cell ALL and lymphoblastic lymphoma represent one such challenge, where finding an appropriate target antigen is debated.[102] Finding novel targets is a challenge, especially if there is a lack of tumour-specific antigens, resulting in the potential for off-tumour, on-target toxicities, if single antigens are targeted or CAR-T cell exhaustion if targeted antigens are widely expressed. Even in the setting of optimal targets, the immunosuppressive tumour microenvironment can render highly potent CAR-T cells ineffective, and in these situations, CAR-T cell therapy may need to be delivered alongside multiplexed, immune-modulatory therapies, or be "armoured" to withstand adverse immune-regulation.

Similar obstacles are faced finding optimal CAR-T cell constructs for targeting acute myeloid leukaemia (AML). AML, a phenotypically diverse disease, represents a bigger challenge as AML blasts share common antigens with normal myeloid cells and haematopoietic stem cells (HSCs). Strategies to mitigate off-tumour on-target effects include multi-targeting CARs employing sophisticated gating strategies to selectively recognise AML blasts or by editing HSCs to prevent expression of CAR-targeted antigens.[103,104] Finding an optimal CAR-T therapy to treat AML, and improve outcomes of this disorder for both children and adults with this disease, is a key goal of ours, working alongside mentors and colleagues including Dr Martin Pule and Prof Persis Amrolia, as well as of other UK investigators. For example, Prof Waseem Qasim is soon to launch a clinical study of off-the-shelf universal CAR-T cells targeting a range of AML antigens (the CARAML study).

Standing on the shoulders of giants in the UK in the fields of adoptive therapy and stem cell transplantation, we hope that in the next 60 years of British haematology, we will be closer to the dream of every stem cell transplant physician: delivering high-precision adoptive cancer therapies with minimal side-effects.[105–107]

Conflicts of interest

Sara Ghorashian received honoraria from Novartis and patents and royalties from UCL Business. Srdan Rogosic has no conflicts of interest to declare.

Author Contributions

SR and SG wrote, edited and read and approved the final manuscript.

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