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A novel immunotherapy — the history of CAR T-cell therapy

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ABSTRACT

Robust research over the past 30 years has led recently to the first approval of genetically enhanced T lymphocytes expressing chimeric antigen receptors (CAR T-cells) as a tool to fight cancer. The backbone of the aforementioned therapy is to equip patients' T lymphocytes in a genetically modified receptor that can recognise the antigen present on the surface of a cancer cell with the accuracy of a specific antibody, and to ignite a cytotoxic reaction against it with the function of the T-lymphocyte receptor. Ground-breaking results achieved in patients with haematological malignancies led to multiple clinical trials of CAR T-cell-based therapy in solid tumours. Regardless of the initial hurdles, recent reports suggest that continuous evolution and further improvements of CAR T-cell therapy for solid tumours is as successful as that observed in haematology. Despite the fact that enormous efforts are still to be made, implementation of CAR T-cells into the clinical oncologist's daily routine practice was never as plausible as it is today.

Key words: personalised medicine, genetic modifications, CAR T-cell therapy, solid tumours, haematological malignancies

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Introduction

Over the years, different areas of cancer biology have been explored to find a cure for cancer, the disease with complex, advanced mechanisms that can easily outsmart the best research teams despite their enormous efforts. Only a few studies every year have succeeded to provide a regimen significantly improving the survival of cancer patients. There was an urgency to search other versatile and intelligent approaches for a more effective fight against cancer. The very best field to exploit appeared to be immunotherapy and enhancing the function of patients' own immunological system by equipping its immunocompetent cells with additional functions to independently combat malignant cells.

By altering immunologic response against cancer cells researchers seemed to significantly improve the outcomes in comparison with standard systemic chemotherapy. Immunologic response can be guided in various ways, and the basic studies in that area were rewarded with the Nobel Prize this year, providing a backbone for

the discovery of checkpoint inhibitors, e.g. ipilimumab, pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab, which have already been successfully implemented into clinical practice. The other approach is directed at increasing the number of tumour infiltrating lymphocytes (TILs), the concentration of which in solid tumours and surrounding stroma is known as a good prognostic factor [1]. The last and the most advanced area of cancer immunotherapy is genetic engineering of patients' immunocompetent cells to produce clones that can act more effectively and accurately, and this area will be discussed in this publication.

The idea of CAR T-cell therapy

Chimeric antigen receptor T-cells (CAR T-cells) are T-lymphocytes genetically modified to express on their surface powerful receptors with enhanced ability to effectively attack cancer cells. Normal T-lymphocytes are unable to fight cancer effectively because they require

major histocompatibility complex (MHC) class I/II antigen recognition to ignite their reaction, and cancer cells deliberately inhibit MHC expression on their surface to be “invisible” to immunocompetent cells. The main concept is to equip patient’s T-lymphocytes with additional functionalities to improve recognition, trafficking, and action against cancer cells. Genetic alterations result in creating T-cell receptor (TCR) with an extracellular domain substituted by a fragment of a specific antibody against cancer antigen (scFv). In this way we can combine both of its functions in one chimeric protein: the ability to trigger T-lymphocyte cytotoxic reaction and to recognise with the accuracy of an antibody a chosen antigen on the surface of a malignant cell without the need for MHC class I/II recognition. Additionally, it is known that adding further co-stimulators to CAR protein can prolong T-cell viability and enhance cytotoxic reaction, among other functions [1, 2].

Surprisingly, the very idea of genetically modified receptors on the surface of immunocompetent cells is not recent. The first report on chimeric combination of receptors and antibodies was published in 1989 by Weizmann Institute in Israel [3]. Since then a great amount of effort has been devoted into this area of research, leading to therapeutic success in 2012 when seven-year-old Emily Whitehead was cured from relapsed/refractory B-cell acute lymphoblastic leukaemia (R/R B-ALL) with infusion of anti-CD19 CAR T-cells. CD19 is an example of the ideal antigen for CAR T-cell recognition because it is expressed exceptionally on every B lymphocyte as well as on blast cells that originate from the B-cell line. Her case was a breakthrough not only because she was the first patient with R/R B-ALL, who achieved complete remission after a single course of treatment, but also because she was the very first child enrolled into a clinical study with tisagenlecleucel (Kymriah, Novartis) CAR T-cell therapy. Her case was broadcasted worldwide as an example of this miraculous drug, with headlines playfully reporting the girl cured from cancer by HIV (actually, the HIV virus was used only as a vector in the transduction process) [4]. At the time of writing this manuscript she is still in complete remission advocating in favour of implementing wider access to CAR T-cell therapy.

Manufacturing process

To start to produce CAR T-cells eligible for administration to the patient, a labour-intensive process that requires the cooperation of both clinical and laboratory staff must be undertaken with many carefully performed steps. Firstly, viable T-cells need to be collected from peripheral blood or through leukapheresis. Next, Th17 lymphocytes are filtered and their gross number

increased by enforcing T-cell multiplication *ex vivo*. Then a previously prepared viral vector containing genetic information about what the future chimera of the antibody and receptor should look like and what kind of antigen it should recognise transports the information into the infected T-cell. For the transfection process several viruses (especially lentivirus or retrovirus) or plasmids are used. Of course, the viral genome must be altered to silence its own virulence after transfection [5]. As for vector production improvement, a highly accurate and efficient CRISPR/cas9 endonuclease system, as well as a TALEN gene-editing tool, finds their implication, making the production process far more precise and increasing the throughput [6, 7]. The basis of the transfection process is to incorporate the message into the T-lymphocyte genome to enforce expression of numerous functional CARs across its membrane.

Solution of transfected T-cells with high expression of CARs on their surface is further expanded in a cell culture, washed, suspended in a mixture of DMSO/dextran 40/HSA/dextrose/Plasma-Lyte A, and cryopreserved. Sterility tests are conducted before shipping to the facility where it will be administered to the patient [8, 9].

Clinical applications in haematological malignancies

Administration requires premedication with paracetamol and H1-antihistamine. Regarding dosing, there are different ranges of total viable CAR T-cells, which vary between children and adults with numbers between 0.2×10^6 and 6.0×10^8 per kilogram body weight CAR-positive T-cells for Kymriah, and 2×10^6 CAR T-cells per kilogram body weight for Yescarta (trade name for axicabtagene ciloleucel, Gilead, approved for treatment of R/R large B-cell lymphoma). Calculated total number of cells is later infused over three to four doses administered with short breaks one after another [8, 9]. Prior to the infusion the patient must undergo lymphodepleting chemotherapy (fludarabine and cyclophosphamide or equivalent) provided that his/her white blood cell (WBC) count is higher than $1 \times 10^9/L$. CAR T-cell infusion must be administered between the second and 14th day after completion of the lymphodepleting chemotherapy [9].

There are many limitations of this treatment. Apart from limited availability of the technology and economic factors, patient specific eligibility criteria must be fulfilled. At the moment of publication FDA registration applies to patients with R/R B-cell ALL and adults with R/R B-cell lymphomas (Table 1). However, efforts are being made to expand those indications for follicular lymphoma (FL) and chronic lymphoblastic leukaemia

Table 1. FDA-approved indications for both tisagenlecleucel and axicabtagene ciloleucel

Generic name	Brand name	FDA approval date	Indications
Tisagenlecleucel	Kymriah, Novartis	August 30, 2017	For patients up to 25 years of age with B-cell precursor acute lymphoblastic leukaemia (ALL) that is refractory or in second or later relapse [9]
		May 1, 2018	For adult patients with R/R large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high-grade B-cell lymphoma and DLBCL arising from follicular lymphoma [9]
Axicabtagene ciloleucel	Yescarta, Gilead	October 18, 2017	For adult patients with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma [8]

(CLL). Last but not least, the patient must be able to have his/her lymphocytes harvested, which excludes cases with deep lymphopaenia (less than 300/ μ L). Viral infections, e.g. HIV, HCV, or HBV, excludes patients from enrolment, as well as active autoimmune disease requiring immunosuppressive therapy. Candidates must also be fit for conditioning chemotherapy with cyclophosphamide and fludarabine or equivalent prior to CAR T-cell infusion with the baseline ECOG performance status of 0–1 [8, 9].

Anticipated adverse events

Unluckily, serious adverse events grade 3 or higher occur in the vast majority of patients treated with Kymriah or Yescarta. Based on the ELIANA and JULIET trials for Kymriah and ZUMA-1 for Yescarta we can assess their incidence as 83% for B-lymphocyte aplasia, 49% for cytokine release syndrome (CRS), 37% for febrile neutropaenia, 22% for hypotension, 18% for hypoxia, 15% for pyrexia, 15% for acute kidney injury, 10% for encephalopathy, and 10% for pulmonary oedema, among others [9].

CRS and neuro toxicities are most life-threatening side effects associated with CAR T-cells infusion. CRS arises from activation of CAR T-cells and death of targeted cells after antigen recognition and TNF α , IL-6 and IFN γ release among others, triggering an avalanche of reactions, which is unlikely to limit itself. The syndrome manifests with fever, hypotension, hypoxia, and tachycardia and can be associated with multiple organ failure and coagulopathy. Severity of CRS is known to correlate with tumour burden. It was observed that fractionation of the infusion volume into 3–4 smaller portions may decrease the risk of CRS. It usually occurs 2–3 days after infusion and lasts for approximately eight days if treated [10].

The majority of neurologic toxicities, e.g. delirium, aphasia, seizures, and encephalopathy, are thought to be

reversible; however, the mechanism of central nervous system involvement is not fully understood. Neuro toxicities grade 3 or higher occurred in 31% of patients with median time to onset of four days and median duration of 17 days. There were four deaths related to Yescarta and one to Kymriah reported in the aforementioned studies, all of them due to CRS [8, 9].

One of the natural side effects of CAR T-cell anti-CD19 therapy is B-lymphocyte aplasia. It was proven that some of B-lymphocytes can lack CD19 expression and flee this way from CAR T-cell activity sustaining baseline immunocompetence, although the majority of patients require replacement therapy with intravenous immunoglobulin and prophylactic antibiotics [1, 5]. Aplasia is thought to be a long-lasting side effect that is present at six months after the treatment in 83% of patients (95% CI 69–91%) [11].

Management of side effects

Management of side effects requires standard symptomatic treatment, although for CRS and neuro-toxicities grade 2 or higher, administration of tocilizumab alone or with corticosteroids is recommended as well [8, 9]. Tocilizumab is an immunosuppressive drug inhibiting specifically IL-6, widely available in Poland in the therapy of rheumatoid arthritis (RoActemra, Roche). The US Food and Drug Administration (FDA) approved tocilizumab for treatment of CRS triggered by CAR T-cell therapy. It is suggested the administration of 8 mg/kg intravenously over one hour repeating every eight hours if needed. A maximum of three doses in a 24-hour period can be administered with a total of four doses [8, 9]. If there is no improvement within 24 hours after starting tocilizumab, administration of corticosteroids as well, preferably methylprednisolone 1 mg/kg intravenously twice a day or dexamethasone 10 mg every six hours, is recommended [8, 9].

Clinical trials

Based on clinical trial data that led to Yescarta and Kymriah FDA approval for adults with R/R B-cell lymphoma (ZUMA-1 and JULIET study), we acknowledge the progression-free survival (PFS) rate at 15 months to be 41% (95% CI 31–50), median duration of response to be 11.1 months (95% CI 3.9 to could not be estimated), the median PFS to be 5.8 months (95% CI 3.3 to could not be estimated), and OS rates of 52% at 18 months with median overall survival (OS) not reached (95% CI 12.0 months to could not be estimated) [12].

For Kymriah, in the ELIANA study of 75 patients not older than 21 years with R/R B-cell ALL the overall remission rate within three months was 81%, the rates of event-free survival and OS were 73% (95% CI 60–82) and 90% (95% CI 81–95), respectively, at six months and 50% (95% CI 35–64) and 76% (95% CI 63–86) at 12 months of follow-up [11].

Worth mentioning is the third, still ongoing, trial — TRANSCEND NHL-001 in R/R aggressive non-Hodgkin lymphomas (DLBCL, CLL, MZL, PMBCL, FL) with lisocabtagene maraleucel (liso-cel, Celgene). It reported an overall response rate (ORR) of 74%, and complete remission (CR) of 52% with only 1% and 15% of grade 3 or higher CRS and neuro toxicity, respectively, which seems to be highly promising compared with the data on Kymriah and Yescarta [13]. The other study of interest described the efficacy of CAR T-cells targeting B-cell maturation antigen (BCMA) expressed highly on multiple myeloma malignant cells. Twenty-one patients were reported to be treated with bb2121 (anti-BCMA CAR T-cells), with an ORR of 89% and follow-up ranging from 1.4 to 54.4 weeks, with only one progression among 21 heavily pre-treated patients [14].

Availability

Although CAR T-cell therapy is undoubtedly highly effective, it is not available outside clinical trials and private health care system. The majority of clinical trials are carried on at facilities in China and in the USA, with the University of Pennsylvania being the leading one. In Europe, the only institutions having some experience with CAR T-cell clinical trials are in the Netherlands and in the UK [1, 15].

Due to cost concerns the UK's NHS initially rejected in August 2018 broad access to Gilead's Yescarta, although the application sparked further discussion. Finally, late September 2018 brought an agreement that resulted in the founding by the NHS of a treatment programme with Yescarta for 200 adult patients with R/R large B-cell lymphoma a year and with Kymriah for 30 R/R B-ALL children and young adults a year. This

precedence makes the UK the first country in Europe offering, still to a limited number of patients, these novel and highly promising therapies.

Apart from clinical trials, several institutions offer private access to CAR T-cell therapy with costs fully covered by patients, with Israel and the USA being the leading ones. Yescarta and Kymriah cost \$373,000 and \$475,000, respectively. An NHS report last year, which summarised costs of treatment with Yescarta jointly with costs of conditioning therapy, hospitalisation, adverse event management, and follow-up, estimated the total cost at £583,362 compared with £80,106 for standard of care [16]. However, contrasting opinions seem to appear recently in peer-reviewed journals assessing life-years gained and quality-adjusted life-years (QALYs) gained in favour of Kymriah vs. standard care. In cases of childhood R/R B-ALL, 40% of patients treated with Kymriah are expected to be long-term survivors with life-years gained of 10.34 years and 9.28 QALYs gained vs. 2.43 years and 2.10 QALYs gained for clofarabine treatment, in comparison. These enormous differences result in a cumulative cost-effectiveness ratio of \$46,000 per QALY gained between Kymriah and clofarabine [17].

Potential in solid tumours

Translation of CAR T-cell success in haematology into the treatment of solid tumours is highly challenging due to many features of solid tumours that in haematological malignancies are minor obstacles. Using genetically modified lymphocytes to combat blasts that share haematopoietic origin and have the potential to migrate through the same locations, like blood, bone marrow, or lymph nodes, might contribute to anti-CD19 CAR T-cell therapy success. Due to genetic instability (somatic mutations) and heterogeneity, cancer cells have variable antigen expression levels on the surface of the cell between subclones of cancer cells. Additionally, antigens expressed by solid tumours are not exclusive comparing with healthy cells, being the foundation of serious “on-target off-tumour” side effects that limit its application [5]. Choosing an ideal tumour antigen (present on every malignant cell and not expressed on the surface of healthy ones) to be targeted by CAR T-cells seems to be the biggest obstacle. Many candidate antigens were under the scope, e.g. MUC1 [18, 19], HER2 [20], G2D [2], CEA [5], EGFR [5], GP100 [21], and mesothelin [2] among many others [22]. As an example, prostate-specific membrane antigen (PSMA) seems to be the perfect target, because preliminary data report it can be found on malignant prostate cells and the endothelium of some tumour vasculature, but it is not expressed by normal cells [2].

The other obstacles are immunosuppressive properties of surrounding stroma that mute activation of the immune system. Sadly, T-cells do not infiltrate tumour tissue easily, and efforts are being made to implement additional receptors and co-stimulators into the CAR T-cell membrane to simplify its trafficking, as well as altering the chemokine secretion profile of the CAR T-cell to correlate with the cancer cells [2, 7]. Surprisingly, the addition of anti-PD-1 antibody appeared to decrease the myeloid-derived suppressor cell (MDSC) population in the tumour stroma, and it augments the response rate through increased CAR T-cell anti-tumour activity [5, 23].

Case series

Sadly, there are only a few case reports and trials on CAR T-cell in solid tumours. There are publications reporting CAR T-cell usage in patients diagnosed with malignant pleural mesothelioma [18], pancreatic ductal adenocarcinoma [18], colorectal adenocarcinoma [2, 24], prostate cancer [2], breast cancer [25], melanoma [21], or osteosarcoma [20] among others. The majority of authors report poor outcomes of the treatment with rare and short-lasting ORR and occasional CR, mostly in melanoma cases [21]. A large number of clinical trials are still recruiting, and more data on clinical effectiveness of CAR T-cell therapy in solid tumours are to be anticipated in near future.

A ground-breaking case report of a female patient with chemorefractory metastatic breast cancer achieving CR after infusion of genetically modified T lymphocytes at the National Cancer Institute in Bethesda, USA was published in June 2018. Interestingly, researchers created a suspension of four different T-cell clones directed against the four highest expressed antigens on the surface of the patient's cancer cells. After myeloablation therapy and infusion of modified autologous T-lymphocytes she continued pembrolizumab as a maintenance therapy and achieved CR after a year of treatment, and sustained it for 22 months of follow-up [25].

New ideas

Because CAR T-cell therapy, apart from its ground-breaking effectiveness, has some serious flaws, efforts are being made to alternate the original idea in order to overcome its limitations, e.g. serious and common side effects, robust manufacturing process, high costs. Studies are ongoing in both the public and private sector exploring different approaches to reach improvement.

Using natural killer (NK) lymphocytes instead of T-lymphocytes for gene editing emerged as one of the

major initiatives. The main advantage brought by the use of NK lymphocytes is that they ignite their activity regardless of human leukocyte antigen (HLA) matching and for that reason do not need to be harvested from a patient or HLA-matched donor. This distinctive feature makes CAR NK cell-based therapy an "off-the-shelf" resource for cancer therapy in contrast with CAR T-cells, which are highly personalised and produced specifically "for-the-patient".

The concept of creating CARs on the surface of NK-lymphocyte is not entirely new. NK-lymphocytes obtained from pooled peripheral or cord blood, as well as from cell line NK92, were previously genetically altered. In the case of NK92 cell line, to prevent permanent engraftment of NK92 cells harvested initially from a non-Hodgkin lymphoma patient, altered lymphocytes needed to be additionally irradiated, but the procedure lowered significantly their viability and ability to proliferate *in vivo* [26]. For all the aforementioned sources of NK-lymphocytes production process had comparable efficiency as in the case of T-lymphocytes, and, additionally, infusions appeared to be much safer with rare side effects at much lower grades of intensity. Despite having lower toxicity, this therapy, surprisingly, turned out to be ineffective with negligible ORR rates [27].

However, a recent study published data on genetically modified NK-lymphocytes obtained through transduction of genetic information on CARs into immunologically pluripotent stem cells (iPSC) that were afterwards forced to transform into NK lymphocytes expressing CAR. The process was described as extremely efficient with a high count of viable CAR NK-cells harvested. Ovarian cancer mice models were then infused with suspension of CAR NK-cells, among others, for comparison showing substantial and long-lasting regression of the tumour volume, proving its superiority above modified CAR T-cell therapy in this case [28].

CAR T cells are forced to express modified CAR particles among endogenous T-cell lymphocyte receptors (TCR), receptors that are recognised as patient specific, and that is the main reason restricting it from being used in an "off-the-shelf" manner. Another example of an approach to overcome this obstacle is to genetically silence expression of native TCR on CAR T-cells that could be given regardless of HLA compatibility with no risk of triggering graft vs. host disease. Several companies made efforts in this area of research, as well as equipping CAR T-cells in suicide genes or other co-stimulatory particles that could add improvements if needed [2, 29]. Apart from large pharmaceutical companies, biotechnology businesses like Cellectis, Parker Institute for Cancer Immunotherapy, or TMunity and others spread over the UK, Australia, China, or Singapore could be the best examples of attempts to commercialise CAR T-cell therapies through the aforementioned improvements.

Conclusions

CAR T-cell therapy is considered highly innovative and effective, undoubtedly being the biggest breakthrough in cancer treatment in years. However, clinical oncologists need to be aware not only of the obvious virtues of this approach but also its limitations. However, the race to create a cancer cure has not been won yet, and countless research teams are working on the idea of training lymphocytes to highly specifically, safely, and more efficiently deal with cancer cells, which will hopefully evolve into an “off-the-shelf” treatment with a more affordable price for caregivers.

Regardless of further improvements, we should all be prepared for the implementation of therapy with genetically modified lymphocytes in the future. Most importantly, nowadays we also should be able to answer patients’ questions on this breakthrough treatment and inform them about its advantages and disadvantages. Furthermore, we should all be aware of the possible side effects and its management, to be prepared for the moment when we can treat our patients with CAR T-cell therapies.

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