CHAPTER 21

Gene-Editing Technologies in Adoptive T Cell Therapy for Cancer: An Ethical Analysis

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BACKGROUND

For at least 50 years, chemotherapy, radiation therapy and surgery, alone or in combination, have been the three most used cancer treatments. Together with the enormous advances achieved in understanding cancer biology, they have contributed to substantially improved outcomes for patients. Nevertheless, the prognosis of numerous cancers remains poor.

In recent years, a fourth and radically different approach of therapy has started to emerge: immunotherapy, i.e., cancer therapy based on engaging the patient's own immune system.

The idea of using the immune system to treat cancer is not new. The first physician to use an immunological approach was the surgeon William Bradley Coley (1862–1936) (Trapani and Darcy, 2017), who developed a treatment based on provoking an immune response to bacteria. Specifically, Coley developed the theory that postsurgical infections had helped patients to recover better from their cancer by provoking an immune response. In 1891 he started to experiment by deliberately causing this phenomenon, injecting Streptococci directly into patients being treated, but the associated high mortality led to abandon this approach soon (Trapani and Darcy, 2017).

The idea of using immunotherapy in cancer returned to prominence with Lewis Thomas (1913–1993) and Frank Macfarlane Burnet (1899–1985), who proposed the theory of cancer immunosurveillance in 1957 (Corthay, 2014). This theory suggested that the immune system recognised and destroyed clones of transformed cells before growth into clinically evident tumours. However, it took 50 years for this view to gain acceptance.

Another example of using attenuated bacteria to treat malignancies resurfaced in 1976 when a research was carried out for testing the use of a tuberculosis vaccine – the Bacille Calmette-Guérin vaccine – as a means of preventing the recurrence of non-muscle invasive bladder cancer (Targeted Oncology, 2014).

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In more recent years, immunotherapy has become an important part of treating some types of cancer. Immunotherapy includes treatments that work in different ways. Some boost the body's immune system in a very general way. Others help train the immune system to attack cancer cells specifically. Examples of immunotherapy now used to treat cancer are monoclonal antibodies or cancer vaccines.

Only few years ago, a new and very promising form of immunotherapy has emerged, i.e., adoptive T cell therapy. It uses genetically modified living autologous or allogenic immune cells as a 'living drug' (Sadelain et al., 2013) to rally the patient's own immune system to destroy cancer cells. Such modified T cells have demonstrated great promise in clinical trials and led to important results in patients with some types of liquid cancer.

However, the current standard treatment requires autologous adoptive cell transfer, which is rather expansive and time-consuming. To generate universal and more potent modified T cells, gene-editing technologies have recently been applied, the most novel and powerful of which is the well-known clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (CRISPR/Cas9) system, an RNA-guided DNA targeting technology.

AIM

The aim of this chapter is to discuss the use of these advances in immunotherapies for cancer from an ethical standpoint. The analysis will be restricted to the application of gene-editing technologies to adoptive T cell immunotherapy.

MATERIALS AND METHODS

As well-known, within the field of (bio)ethical research, a considerable number of methods, models and frameworks can be successfully used to conduct ethical analyses. The present chapter will be carried out in the light of the four well-known moral principles of beneficence/nonmaleficence, autonomy and justice (Beauchamp and Childress, 2008).

Information was gathered through literature search as well as philosophical analysis of the logic and coherence of the argumentations. A comprehensive literature search was conducted using the PubMed-NCBI database.

The result reported does not explicitly include all the steps of the analysis performed. The main findings have been summarised and organised into the paragraph 'Findings' (see below).

TECHNOLOGY DESCRIPTION

To conduct ethical analyses, the first step to perform is to collect the main 'facts'. In the present work, this activity is equivalent to describing the technologies in question. Below is thus presented a brief description of the technologies under investigation.

Adoptive T Cell therapy

Adoptive T cell therapy is a type of immunotherapy that consists in isolating lymphocytes for stimulating and expanding ex vivo antigen-specific T cells that are subsequently infused into a patient to treat a cancer (Gomes-Silva and Ramos, 2018). T cells recognise tumour antigens through their antigen receptor (T cell receptor) allowing them to produce a specific immune response potentially able of eliminating tumours in a short period of time (Gomes-Silva and Ramos, 2018). Because T cells have the effector or memory functions of the normal immune system, they have also the capacity to control tumours for a much longer time in the patient's body than traditional drugs. For this reason they are also called 'living drugs' and are often compared to vaccines (Li and Zhao, 2017).

The ability of T cells to mediate anticancer effects was first demonstrated in a series of researches both in vitro and in vivo between the 1950s and 1960s (Mitchison, 1955; Barnes and Loutit, 1957; Mathe et al., 1965). Since then, unleashing the T cell compartment has become the focus of numerous immunotherapy cancer researches (Singh et al., 2017). In the last years, the most exciting results have been achieved by the genetic modification of T cells with chimeric antigen receptors (CARs) (CAR-T cells, initially referred to also as T-bodies) (June et al., 2012).

CARs are 'engineered membrane proteins that consist of three main components: an extracellular antigen-recognition region, a hinge and transmembrane region and an intracellular T cell activation region' (Li and Zhao, 2017).

Three generations of CAR-T cells have already been developed, based on increasingly sophisticated architecture. As well explained by Li and Zhao,

> *the first-generation CARs have only the CD3ζ signaling domain, which provides only signal 1 for T cell activation (…) Early clinical trials using first-generation CARs to treat cancers showed very limited responses and the CAR-modified cells persisted at low levels for only weeks-to-months, suggesting first-generation CAR-T cells lack sufficient activation signals to support the long-term T cell expansion that is required for efficient anti-tumour effects. Second-generation CARs were generated by adding a signaling domain derived from a co-stimulation molecule (…). The second-generation CAR-T cells showed an improved capability to expand and persist in vivo, an activity that has been demonstrated in clinical trials (…). Based on second-generation CARs, third-generation CARs contain multiple costimulatory domains in the intracellular signaling domains (…). However, the superior efficacy has not been validated clinically (…).*
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> ***Li and Zhao, 2017***

Most of the ongoing clinical trials are using second-generation CARs. The encouraging results have attracted the interest of pharmaceutical and biotech companies. Thus, in 2017, two CAR-T cell therapies were first approved by US Food and Drug Administration (FDA): the first one (approved on August 30, 2017) is tisagenlecleucel (Kymriah; Novartis, Basel, Switzerland) for the treatment of paediatric and young adult patients (aged up to 25 years) with relapsed and/or refractory B-cell precursor acute lymphoblastic leukaemia after at least two prior lines of treatment (US Food and Drug Administration, 2017a); the

second one (approved on October 18, 2017) is axicabtagene ciloleucel (Yescarta; Kite Pharma, Santa Monica, CA, USA) for the treatment of 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 (US Food and Drug Administration, 2017b).

Both products are manufactured in a stepwise way: the T cells are collected by a process called 'leukapheresis', shipped to a manufacturing centre, where they undergo genetic transduction, are shipped back to the hospital and subsequently infused into the patient (Prasad, 2018). Because of the complex logistics of their production, these therapies are administered only at specialised cancer centres and have a high cost of manufacturing as well as a high price (Kymriah: $475,000; Yescarta: $373,000, Institute for Clinical and Economic Review, 2018).

A great challenge remains to boost a potent cellular immune response to eradicate not only blood cancers but also solid tumours, which so far have been much more difficult to treat. Anyway, several CAR-T therapies targeting solid cancers antigens, such as Her2/neu, Mesothelin cMet, GD2, interleukin-13 receptor alpha 2 (IL13Rα2), CEA and EGFR, are currently under evaluation in different phases of clinical trials (Ren and Zhao, 2017). T cell engineering may thus extend the success observed in haematologic cancers to solid tumours. What is more, other cell types are considered beyond the more common αβ T cells to achieve cancer remission, including the rarer γδ-T cell subtype, as well regulatory T cells (Tregs), natural killer cells, red blood cells, macrophages and even nonimmune cells (Di Marco Barros et al., 2016; Sebestyen et al., 2016; Villa et al., 2017; Elena et al., 2015; Kojima et al., 2018).

Genome-Editing Technologies in Cancer Immunotherapy

As mentioned, CAR-T cell therapies currently mainly use autologous T cells collected from patients; these new treatments have shown exciting results with up to 70% complete remission, which however is not yet synonymous to a full curative effect (Porter et al., 2015; Neelapu et al., 2017; Neelapu, 2017; Locke et al., 2017). However, this approach has some important limitations too: for some patients, cell manufacturing is not feasible or fails due to poor lymphocyte counts, poor T cell quality or too high a tumour burden, which has been positively correlated with a dramatically increased incidence in fatal cytokine release syndromes; furthermore, cell manufacturing is long and very expensive (Singh et al., 2017).

For these reasons, much effort has lately been carried out to produce the so-called 'off-the-self' CAR-T cells (also named 'universal CAR-T cells'), generated from healthy donors to treat multiple patients. Such allogeneic cell-based therapies could solve the problem of poor T cell collections and long-manufacturing delays (Singh et al., 2017).

In turn, the greatest technical barrier to implement a third party adoptive therapy is the prevention of Graft-versus-Host Disease (GvHD). To overcome this barrier, researchers have started to use gene-editing technologies (Singh et al., 2017; Ren and Zhao, 2017; Gautron et al., 2017; Zhang et al., 2017; Mussolino et al., 2017; Ren et al., 2017a,b; Rupp et al., 2017; Sadelain, March 2, 2017; Qasim et al., 2017; Liu et al., 2017), specifically, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and the CRISPR–Cas9 system (Singh et al., 2017; Ren and Zhao, 2017; Gautron et al., 2017; Zhang et al., 2017).

ZFNs, TALENs and the CRISPR–Cas9 system are recent technologies for making precisely targeted alterations to DNA sequences in living cells.

Techniques to modify DNA in the genome exist since the 1970s. The earliest method – developed in the late 1970s – that researchers used to edit genomes in living cells was homologous recombination. Homologous recombination refers to the exchange (recombination) of genetic information between two similar (homologous) strands of DNA. However, despite this technique being extremely useful to engineer microorganisms, it has several limitations, and particularly its inefficiency in most mammalian cell types and high rate of errors, causing the so-called 'off-target edits'.

Other approaches have since been developed. In the 1990s scientists started to use ZFNs, artificial restriction enzymes generated by fusing a zinc-finger DNA-binding domain to a DNA-cleavage domain (Kim et al., 1996). The structures of ZFNs were engineered from naturally occurring proteins that were discovered in eukaryotic organisms. Although ZFNs improved the success rate of genome editing and have been successfully deployed at the commercial scale, their application remained expensive and time-consuming.

In 2009, a new class of proteins called TALENs emerged in the genome-editing scene (Christian et al., 2010). Similar to ZFNs, TALENs were engineered from proteins found in nature and were capable of binding to specific DNA sequences. Although TALENs and ZFNs were comparable in terms of efficiency, TALENs offered the advantage of greater simplicity (Christian et al., 2010).

Cas9 endonuclease technology is the most recently developed gene-editing platform. Unlike its predecessors, CRISPR is a simple technology with little assembly required. In 2012, it was discovered that a system of defence against viral attack found in the bacterium *Streptococcus pyogenes* could be adapted as a programmable system for genome editing (Jinek et al., 2012). The system consists of two key molecules that introduce a change (mutation) into the DNA: an enzyme called 'Cas9', and a piece of RNA called 'guide RNA' (gRNA). The gRNA is designed to find and bind to a specific sequence in the DNA. The Cas9 follows the gRNA to the same location in the DNA sequence and makes a cut across both strands of the DNA. At this stage the cell 'recognises' that the DNA is damaged and tries to repair it.

This system has brought major improvements to the speed, cost, accuracy and efficiency of genome editing and has generated a lot of excitement in the scientific community (Refolo et al., 2017). It has already been, to varying degrees, applied to many aspects of molecular biology and translational therapy. Many of these applications involve the engineering of the genomes of somatic (nonreproductive) cells, but there is of interest in and debate about the potential to edit germ line (reproductive) cells (Harris, 2015).

In recent times, ZFNs, TALENs and CRISPR–Cas9 system have started to be applied to adoptive T cell therapy too. As mentioned, gene-editing technologies could be used to prevent unwanted GvHD for generating universal CAR-T cells. In addition, they could be used to enhance CAR-T cell function by ablating the genes encoding T cell inhibitory receptors or signalling molecules. Further applications are under investigation (Singh et al., 2017). As noted by Singh et al., the primary advantage is 'site-specific integration of the CAR gene, which eliminates the possibility of an integration event that leads to disruption of an essential gene, as well as endogenous promoter-driven expression' (Singh et al., 2017).

The number of preclinical studies using these technologies is steadily increasing, and several trials are ongoing, whose results will be available in the next few years. Thus, the combination of CAR-T cell technology with gene-editing technologies seems to have inaugurated a new and promising era in cancer immunotherapy.

FINDINGS

As mentioned, the present analysis has been carried out in the light of the four well-known moral principles of beneficence/nonmaleficence, autonomy and justice. The result reported does not explicitly include all the steps of the analysis. The main findings have been summarised and organised into the paragraphs below.

Principle of Beneficence/Nonmaleficence

When the ethical consequences of implementing or not implementing a given health technology are evaluated, the first step is to consider risks (principle of nonmaleficence) and benefits (principle of beneficence) for patients. The term 'risk' refers to the possibility that harm may occur, whereas the term 'benefit' refers to something of positive value related to health or welfare. The dimension of benefits is mainly determined by the therapeutic efficacy, e.g., improvement of a disease, whereas the dimension of risks comprises the safety profile of a drug, including identified adverse drug reactions.

Thus, people are treated in an ethical manner by protecting them from harms, but also by making efforts to secure their well-being including enhanced quality of life. When a technology is implemented, the amount of benefits should outweigh the amount of risks. Only if there is a favourable risk–benefit ratio, the use of a certain healthcare technology can be considered as ethically justified.

Benefit–risk assessment is at the heart of drug approval decisions by regulatory agencies, such as the European Medicines Agency (EMA) and the US FDA. Specifically, within the European Union, the marketing authorisation is supposed to be refused if the benefit–risk balance of the drug is not considered to be favourable, or if therapeutic efficacy is insufficiently substantiated; in the United States drugs have to be effective and safe to receive approval, whereby safe means that the benefits outweigh the risks (Beauchamp and Childress, 2008).

However, the calculation and weighting of risks and benefits is often difficult, and to determine the benefit–risk balance regulators have to investigate a vast amount of data, including quality, safety and efficacy data. Usually, safety is tested in preclinical and clinical trials, while efficacy is mainly examined in phase II and III clinical trials.

In reference to their eventual marketing authorisation process, therapies using T cells edited with gene-editing technologies would be classified as 'advanced therapy medicinal products' (ATMPs).

Legally, the ATMP concept was first introduced in 2003 through the European Directive 2003/63/CE (Harris, 2015; EU directives, 2003) with the aim of indicating a special class of innovative therapies that differ substantially from traditional therapeutic agents. They include gene therapy medicinal products (GTMPs), somatic cell therapy medicinal products (sCTMPs), tissue-engineered products and combined ATMPs.

This introduction was a consequence of rapid advances in life sciences using human biological materials. As explained by Juškevičius,

> *The novelty, complexity, and technical specificity of the application of these advances into medical practice created significant regulatory issues at EU level since a number of cell therapies and engineered tissues have been introduced in some Member States during the last decade. First of all, these novel therapies with high complexity need to be addressed quite differently from traditional pharmaceuticals or biologicals in their development, manufacturing, or administration process (…). Secondly, despite the fact that novel therapies generate huge healthcare expectations and constitute an alternative therapeutic strategy to conventional clinical therapy, for which no effective cure was previously available, at the same time they are expected to bear a higher risk potential than other biological medicinal products not foreseen for transplantation materials such as tumorigenicity, cell (de)differentiation, and patient integration. Thirdly, the novel therapies were subject to different legal regimes: gene therapy, for example, genetic immunotherapy for cancer, and cell therapy, for example, articular chondrocytes for cartilage repair, already had been regulated as medicinal products under the Community legal framework since 2003 while tissue-engineered products, for example, skin replacement materials, remained largely unregulated by EU legislation.*
>
> **Juškevičius, 2013**

Afterwards, in 2007, the issue was addressed in more detail by the European Commission with the introduction of a specific regulatory framework – a *lex specialis*, the Regulation (EC) No. 1394/2007 (EU regulation, 2007), also known as ATMP Regulation – which provided a clear definition of ATMPs, outlined the marketing authorisation requirements and procedures and described the postauthorisation

obligations, specifically focussing on efficacy, safety and risk management (Carvalho et al., 2017).

However, obtaining marketing authorisation process of a gene or a cell therapy product, or an engineered cell therapy product, follows different pathways around the world. For example, in the United States, gene and cell therapies are considered as biological therapies (US Food and Drug Administration, 2018).

In Europe, the EMA has also developed a specific document outlining a risk-based approach for the evaluation of these medicinal products (European Medicines Agency, 2013). Its application in the preparation of a Marketing Authorisation Application dossier is anyway optional, even though, in cases where the risk-based approach is applied, the applicant is advised to follow the methodology proposed.

Like any other drug, ATMPs are subject to the principles of Good Manufacturing Practice, Good Laboratory Practice and Good clinical Practice. However, the clinical use of ATMPs in humans may be associated with specific risks to the patient and to third parties. In turn, these risks are determined by various risk factors, which are related to the quality, biological activity and application of the ATMP. For this reason, the EMA has found it useful to provide a guideline for the identification of risks and associated risk factors of an ATMP and the establishment of a specific profile for each risk.

In the guideline, 'risk' is defined as a 'potential unfavourable effect that can be attributed to the clinical use of ATMP and is of concern to the patient or to other populations (e.g., caregivers and offsprings)' (European Medicines Agency, 2013). Risks associated with the clinical use of ATMPs include for example: 'unwanted immunogenicity, disease transmission, tumour formation, treatment failure, unwanted tissue formation and inadvertent germ line transduction, as well as toxicity due to degradation or leaching of toxic compounds from structural components, due to unintended alteration of cell homeostasis, due to unwanted targeting of cells or organs, due to deregulated therapeutic gene expression and due to contaminants from the production process' (European Medicines Agency, 2013).

On the other hand, 'risk factor' is defined as a 'qualitative or quantitative characteristic that contributes to a specific risk following handling or administration of an ATMP' (European Medicines Agency, 2013). Examples of risk factors that can be associated with cell-based medicinal products could include, but may not be limited to, 'the origin of cells or tissues (autologous vs. allogeneic), the ability of cells to proliferate and differentiate, the ability to initiate an immune response (as target or effector), the level of cell manipulation (in vitro/ex vivo expansion/activation, genetic manipulation), aspects of the manufacturing process, noncellular components, the mode of administration (ex vivo perfusion, local, systemic) and the duration of exposure (short to permanent)' (European Medicines Agency, 2013). Furthermore, as noted by the EMA, 'risk factors that can be associated with GTMPs depend on the vector as well as on the transgene expression cassette used, and in the case of a cell-based GTMP also on the cell population to be

genetically modified. Typical risk factors include, but may not be limited to, the potential of the vector for and its extent of chromosomal integration, vector immunogenicity, the capacity of the vector for latency, or reactivation, or mobilisation and its potential for recombination and reassortment and biodistribution to nontarget sites' (European Medicines Agency, 2013). Finally, patient-, disease- and medical procedure-related risk factors (including those associated with administration of the ATMP) may contribute to a specific risk (European Medicines Agency, 2013).

From the information provided it appears that assessing benefit–risk ratio of applications using T cells edited with gene-editing technologies is not an easy task and needs a strict evaluation. Because of the limitations of space, in this chapter, it is not possible to discuss benefit–risk ratio of all existing experiences.

Generally, it can be said that, although this application seems to be attractive, its safety and efficacy have to be proven (Gomes-Silva and Ramos, 2018). Specifically, an important question to consider is to what extent gene-editing technologies induce off-target cleavage events, especially in therapeutic application. Reducing the off-target effects for safe therapeutic application of gene-editing technologies in immunotherapy remains in fact unresolved. Hence, the final products will have to be carefully assessed with regard to chromosomal translocations. In addition, as noted by Mussolino et al., 'The key to a successful gene-editing approach is the effective introduction of the gene-editing tools into the cell type of interest. However, in a clinical context, not only transfer efficacy but also transfer-associated cytotoxicity and genotoxicity are critical' (Mussolino et al., 2017).

As a consequence, even though the therapeutic potential of gene-editing technologies has been demonstrated in many instances, further research is still required.

This is not surprising if one considers that the evaluation itself of approved CAR-T cell therapies such as tisagenlecleucel remains still rather complex. According to the data submitted to the FDA (US Food and Drug Administration, 2017a), the overall remission rate among 63 response-evaluable patients who received this drug in the pivotal multicenter, open-label, single-arm, phase II Eliana trial was 82.5%. On the other hand, serious adverse reactions have been observed in patients (grade 3 or 4 adverse events were noted in 84% of patients), such as cytokine release syndrome.

To sum up, benefit–risk ratio of applications using T cells edited with gene-editing technologies is a complex matter, and it remains still uncertain. Further studies are necessary.

Principle of Autonomy

A second step to consider is patient's autonomy. This aspect is connected to the issue of the informed consent, an essential prerequisite to the beginning of any medical intervention.

Informed consent is a process by which the healthcare provider discloses appropriate information to a competent patient, so that the patient can make a voluntary choice to accept or refuse the treatment. In this sense, informed consent is connected with the principles of autonomy and the issue of self-determination.

To be valid, the informed consent requires that: the individual should have the capacity to make the decision; the patient's choice should be voluntary; should be provided with appropriate information, in a format the patient can understand, regarding the benefits, risks, consequences and alternatives to the proposed treatment and the patient's decision should be accurately documented (Refolo et al., 2012a,b, 2014; Berto et al., 2000; Minacori et al., 2012).

Like any other drug, applications using T cells edited with gene-editing technologies would be subject to current informed consent regulations on medical experimentations and interventions.

Anyway, after the case 'Gelsinger v. University of Pennsylvania', a huge bioethical debate on informed consent and gene therapy has emerged (Gelsinger and Shamoo, 2008; Smith and Byers, 2002). Jesse Gelsinger (1981–99) was the first person publicly identified as having died in a clinical trial for gene therapy. As mentioned by Carvalho et al.,

> *In 1997, at the University of Pennsylvania, a group of investigators developed an AdV (adenovirus) vector that contained a functional copy of the OTC gene. Eighteen patients with OTCD were enrolled in a phase I dose escalating study, which tested six different investigational product doses. The vector was administered through a femoral catheter into the right hepatic artery. In 1999, Jessie Gelsinger was enrolled and allocated to the highest dose cohort. Just 4 days after administration, a strong immune response against the vector was noted and the patient died due to multiorgan failure. Following FDA inspection, the case unraveled major deficiencies in trial conduct, such as failure to report significant safety information to regulatory bodies, inadequate informed consent process, inclusion of ineligible patients and protocol amendment implementation before IRB approval. In addition, researchers' financial interest in positive trial results was pointed out as potential bias.*
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> **Carvalho et al., 2017**

In response to these concerns, the US government agencies and academic institutions strengthened regulatory requirements for gene therapy clinical trials. For example, at the time, it became mandatory to have Drug Safety and Monitoring Boards for early phase studies (Carvalho et al., 2017).

In more recent years, specific guidances related to the consent process for gene transfer trials have been developed. One of them is the *Informed Consent Guidance for Human Gene Transfer Trials subject to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* (National Institutes of Health, 2014) by the US National Institutes of Health that provides suggestions for preparing consent forms for gene transfer studies to investigators, sponsors, institutional review boards and potential patients. Among topics addressed are: (1) communication about the study to potential participants, (2) conflicts of interest, (3) comprehensibility, (4) time for decision-making and (5) assent. Interestingly, the guidance strongly encourages the use of a separate consent form for the gene transfer component of a study in which either additional investigational interventions or standard treatments are also used.

To conclude, generally, applications using T cells edited with gene-editing technologies are subject to current informed consent regulations on medical experimentations and interventions. The related inform consent processes can be supported by specific documents.

Principle of Justice

A third point to consider concerns issues of justice. Within the healthcare context, issues of justice can be divided along two different but related dimensions: access and allocation. Access refers to 'whether people who are – or should be – entitled to healthcare services receive them', whereas allocation refers 'to determining what resources should be devoted to healthcare' (Emanuel, 2000). In turn, a considerable number of methods has been proposed for the just allocation of healthcare resources, including cost-effectiveness, age-base rationing, the prudent insurer method and the fair opportunity method.

At present, as applications using T cells edited with gene-editing technologies have not been commercialised, no specific consideration can be drawn.

However, CAR-T therapy, just like any other form of personalised medicine, is at the centre of a debate about its economic sustainability. For instance, after approval, Novartis announced that tisagenlecleucel would cost US$ 475.000 for a one-time infusion, a figure that has drawn criticism. This figure does not include the costs associated with leukapheresis, cell infusion and management of complications. In addition, Novartis has announced that the therapy will only be administered at 32 specialised cancer centres in the United States – those with considerable resources and the availability of intensive-care services. Finally, the typical turnaround time for manufacturing of the cells is 22 days (Prasad, 2018). All these aspects raise ethical issues in terms of affordability and distribution of these new innovative and potentially curing drugs.

Certainly, the effective production of universal CAR-T cells by gene-editing technologies would allow to treat a larger number of patients, facilitating access and distribution. Anyway, the economic implications of this application are currently unknown.

Therefore, therapies produced by the application of gene-editing technologies to adoptive T cell immunotherapy will probably raise issues in terms of justice. At present, no specific consideration can be drawn.

PERSPECTIVES

The combination of CAR-T cell technology with gene-editing technologies (such ZFNs, TALENs and CRISPR–Cas9 system) seems to have inaugurated a new and promising era in cancer immunotherapy. The number of preclinical and clinical studies in this sector is steadily increasing, and the related results will be available in the next few years.

From an ethical point of view, in the light of the present analysis, it emerges that: (1) benefit–risk ratio of this combination is complex to evaluate, and it remains still uncertain; (2) applications related to this combination is subject to current informed consent regulations on medical experimentations and interventions, even though informed consent processes can be supported by specific documents; (3) this combination will probably raise issues in terms of justice, but currently no specific consideration can be drawn.

To reach better-founded ethical considerations, it appears evident that further data related to this sector are needed.

However, the present analysis is limited to the researcher's perspective. Actually, implementing a certain health technology has an impact on a wide range of interested parties (stakeholders), such as patients, clinicians, healthcare providers, industrial companies, national/regional authorities and so on. The perspectives of all relevant stakeholders should be thus reflected in any ethical evaluation of health technologies. In turn, taking into account stakeholder perspective is not an easy task because, on the one hand, stakeholder involvement is epistemologically complex (Nielsen et al., 2009); on the other hand, balancing various interests and perspectives can be challenging (Walt, 1994).

For example, if prices of these therapies will remain still so high, balancing interests of payers, public policy makers, patients, industrial companies, etc. will be rather difficult.

As mentioned by Prasad,

> *how much did the CAR-T cell therapy cost to develop and produce? Just days before tisagenlecleucel was approved, the patient-advocacy group Patients For Affordable Drugs issued a letter to the chief executive officer of Novartis, asking that the company price the medication fairly. The letter highlighted the long list of NIH-funded research projects, spanning years, that underpinned the development of CAR-T cell therapies, and estimated that the commercialisation of these treatments has been subsidised by at least $200 million of US taxpayers' money. The group also noted that Novartis obtained licensing rights for tisagenlecleucel from an academic group at the University of Pennsylvania, who developed the CAR-T cell construct, for only $20 million.*
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> ***Prasad, 2018***

The CAR-T cell therapy is not the only example in recent times of treatment with high efficacy but whose costs are unsustainable. Another well-known case is represented by the new direct-acting antiviral drugs for the treatment of patients with chronic hepatitis C. Despite their high efficacy, tolerability and manageability, associated costs were unsustainable (Aronsohn and Jensen, 2012; Kamal-Yanni, 2015; Reau and Jensen, 2014; Simon and Chung, 2015). The introduction of this type of treatment has raised ethical dilemmas and social conflicts, mainly due to the fact that not all patients in need had access to these therapies. The challenge was to guarantee equitable access to these therapies, especially within the context of egalitarian healthcare systems.

Anyway, Bioethics seems not to be still well equipped for addressing such an issue: even though the chapter under which this topic can be categorised (i.e., ethics of

resource allocation in healthcare) is well developed, it seems to be rather theoretical and generic. Therefore, an organic bioethical overview on the topic of equity of access to this type of drugs is to a large extent lacking (Craxì et al., 2016). If this was done, it would perhaps be possible to better analyse and balance the various interests and perspectives.

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