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Review

Modified T-cells (using TCR and CTAs), chimeric antigen receptor (CAR) and other molecular tools in recent gene therapy

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ABSTRACT

T-cell-based cancer immunotherapy by the transfer of cloned TCRs that are isolated from tumor penetrating T-cells becomes a possibility through NY-ESOc259; a human-derived affinity-enhanced TCR that provides a level of sufficiency in long-term safety and efficacy. NY-ESOc259 recognizes a peptide common to CTAs (LAGE-1 and NY-ESO-1) in melanoma. Risks associated with insertion related transformation in gene therapy have been alleviated through strategies that include the engineering of transcription activator like effector nucleases (TALEN), RNA-guided nucleases (CRISPR/Cas9), Zinc-finger nucleases (ZFN). Cancer immunotherapy based on the genetic modification of autologous T-cells (dependent on the engineered autologous CD8+ T-cells), designed to distinguish and destroy cells bearing tumor-specific antigens via a CAR is able to exterminate B-cell leukemias and lymphomas that are resilient to conventional therapies. A tool with a very large reservoir of potentials in molecular therapy strategy is the Pluripotent Stem Cells (PSC), with pluripotency factors that include Klf4, Sox2, c-Myc, Oct4, differentiating into disease-associated cell phenotypes of three germ layers, comprising of mesoderm (e.g. cardiac cells, blood and muscle), endoderm (liver, pancreas) and ectoderm (epidermis, neurons). It finds good application in disease modelling as well as therapeutic options in the restoration of CGD by using AAVS1 as the vector where the therapeutic cassette is integrated into the locus to restore superoxide production in the granulocytes. Fascinatingly, Clinical trial involving iPSC are already underway where scientists have plans to use iPSC-derived cells to treat macular degeneration (a devastating age-related eye disease). Application of these findings has redefined incurable diseases disorders as curable.

Contents

Abbreviations: PID, Primary immunodeficiencies; ADA, Adenosine deaminase; CAR, Chimeric antigen receptors; TCR, T cell receptor; CTAs, Cancer testis antigens; TALEN, Transcription activator like effector nucleases; ZFN, Zinc-finger nucleases; DSB, Double-strand DNA break; NHEJ, Non-homologous end-joining; CCR5, Chemokine receptor type 5; CLL, Chronic lymphocytic leukemia; ALL, Acute lymphoblastic leukemia; PSC, Pluripotent Stem Cells; iPSC, Inducing Pluripotent Stem Cells; LPL, Lipoprotein lipase.

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1. Introduction

Gene therapy (gene replacement, gene editing, and gene insertion) is a reality of one of the many potentials of molecular Biology. A Clear understanding of the molecular mechanisms pivotal to cellular mechanism is necessary for this modification of the blueprint of life towards solving clinical and medical challenges. Though a lot of credit goes to pharmacotherapy, it has not being without associated side effects or adverse reactions. Better sophisticated tools (either new as well as refined or fine-tuned) viral and non-viral vectors, naked oligonucleotides and plasmids have been developed in recent times to solve the clinical challenges associated with gene therapy. Lots of clinical trials have been initiated worldwide (http://www.wiley.com//legacy/wileychi/genmed/clinical/). The central focus of gene therapy is the insertion, deletion, editing/modification of existing portion of the fundamental code (DNA) in varied gene types (Table 1) to produce the desired gene expression outcome, generally targeted at preventing, treating or curing disorders. Early gene therapy with an original focus on Orphan diseases of damaging monogenetic defects that include primary immunodeficiencies (PID), has greatly expanded to cover a number of diverse disorders that now include heart failure, Parkinson's disease, cancers, diabetes, other neurodegenerative and metabolic disorders [1]. These limitations of pharmacotherapy notwithstanding, gene therapy has associated limitations as well, and such include the recognition of the protein on the viral capsid as an antigen by the host immune system. This limitation has been improved on through the more recently designed viral and non-viral vectors, plasmids and RNAs for both *in vivo* and *in vitro* applications. The pioneering motivation for the commencement of the gene therapy trial to repair adenosine deaminase (ADA) deficiency was by using genetically modified T-lymphocytes, creditable to Rosenberg et al., at NCI. Similarly, the first application of genes-modified haematopoietic cells was performed via inserting a bacterial gene into lymphocytes that have the potential of infiltrating cancerous tumors, to track the associated behaviors of melanoma cells when administered into the patients [2]. They are quite a number of successful clinical trials that buttresses the fact that virtually a lot more can also be done through the modification of T-cells such as the incorporation of chimeric antigen receptors (CAR) in order to enhance tumor cell recognition towards facilitating a fleet of cancer killing cells to the target. Gene therapy holds better promises for us in treating diseases such as hemoglobinopathies, cancer immunotherapies, ocular diseases, hemophilia B, neurological diseases [3], immunological, neurodegenerative, hematological and other metabolic disorders that have eluded the cure sought through the conventional pharmacotherapy. Rapid gene therapy improvements are recorded every year (Fig. 1). Different phases of clinical trials (Table 2) are rapidly underway towards improving on the molecular equipment for driving gene therapy to expectation. Progress in enhanced vector systems for *ex vivo* as well as *in vivo* gene transfer have become a true potential for managing various disease with increased range of medical application beyond genetic disorders; redefining diseases previously classified untreatable, curable. There is the need to get the medical and research community updated on the very recent strides in gene therapy through the use of modified T-cells (using TCR and CTAs), Chimeric Antigen Receptor (CAR) and Other Molecular tools, therefore, the need for this study.

Table 1
Gene types transferred in Gene therapy clinical trials in order of ascending percentage hierarchy. Data Sourced from: (www.wiley.co.uk/genmed/clinical) on 2nd June, 2017.

Gene type	Gene Therapy Clinical Trials	
	Number	Percentage (%)
Ribozyme	6	0.2
siRNA	11	0.4
Cell cycle	10	0.4
Hormone	10	0.4
Oncogene regulator	12	0.5
Adhesion molecule	12	0.5
Antisense	17	0.7
Cell protection/Drug resistance	20	0.8
Porins, ion channels, transporters	23	0.9
Transcription factor	35	1.4
Oncolytic virus	52	2.1
Marker	55	2.2
Unknown	63	2.6
Replication inhibitor	94	3.8
Suicide	173	7
Growth factor	176	7.1
Tumor suppressor	181	7.3
Others	199	8.1
Deficiency	206	8.4
Receptor	259	10.5
Cytokine	375	15.2
Antigen	474	19.2
Total	2463	

2. Cancer immunotherapy using TCR and CTAs gene-modified T-Cells

T-cell-based cancer immunotherapy by the transfer of cloned TCRs that are isolated from tumor penetrating T-cells (engineered *ex vivo* to convey their specificity to a specific tumor antigen) becomes a possibility, already demonstrated by a number of gene therapy trials. The engineered tumor-specific T cells are thereafter re-administered to the beneficiary, where they identify tumor antigen in HLAs context in the tumor microenvironment. A variety of cancers including neuroblastoma, synovial cell sarcoma, colorectal cancer and melanoma are treatable with genetically modified TCRs with some level of associated adverse effects that results from off targets [4–6]. Some of the side effects (including neurotoxicity) could be alleviated by finding an alternative to TCRs in Cancer testes antigens (CTAs; MAGE-A3). They are a group of tumor-associated antigens with limited expression to male germ cells (in the testis, trophoblasts, placenta, ovary on some occasions) shared by several sorts of malignances such as bladder, breast, lung, ovary, myeloma, melanoma and metastatic cancer [7,8]. These findings has been supported by a number of current clinical studies with the accession code #NCT01352286; #NCT01350401; #NCT01273181. The fatal adverse effect (mostly neurological) is presumably associated with MAGE-A12 expression and MAGE-A3-specific T-cell infusion [7]. Other adverse effects include severe myocardial damage, raising grave safety concerns. Hope surfaces as NY-ESOc259; a human-derived affinity-enhanced TCR provides a level of sufficiency in long-term safety and efficacy on this approach. NY-ESOc259 recognizes a peptide common to CTAs (LAGE-1 and NY-ESO-1) in melanoma. This finding is supported by clinical studies (#NCT01352286) [9].

2.1. Targeted specific gene correction (amendment) strategies

Though a lot of risks associated with insertion related transformation in gene therapy have been alleviated through the engineering of various vectors, this strategy is not enough, and the continuous input of efforts is necessary to further reduce this risk. These strategies include the engineering of transcription activator like effector nucleases (TALEN), RNA-guided nucleases (CRISPR/Cas9), Zinc-finger nucleases (ZFN) [10–13]. To also achieve this, other ultra-specific site-directed gene correction is employed, and a typical example in the development of genetic scissors via fusing DNA-binding domains unto the catalytic domain of

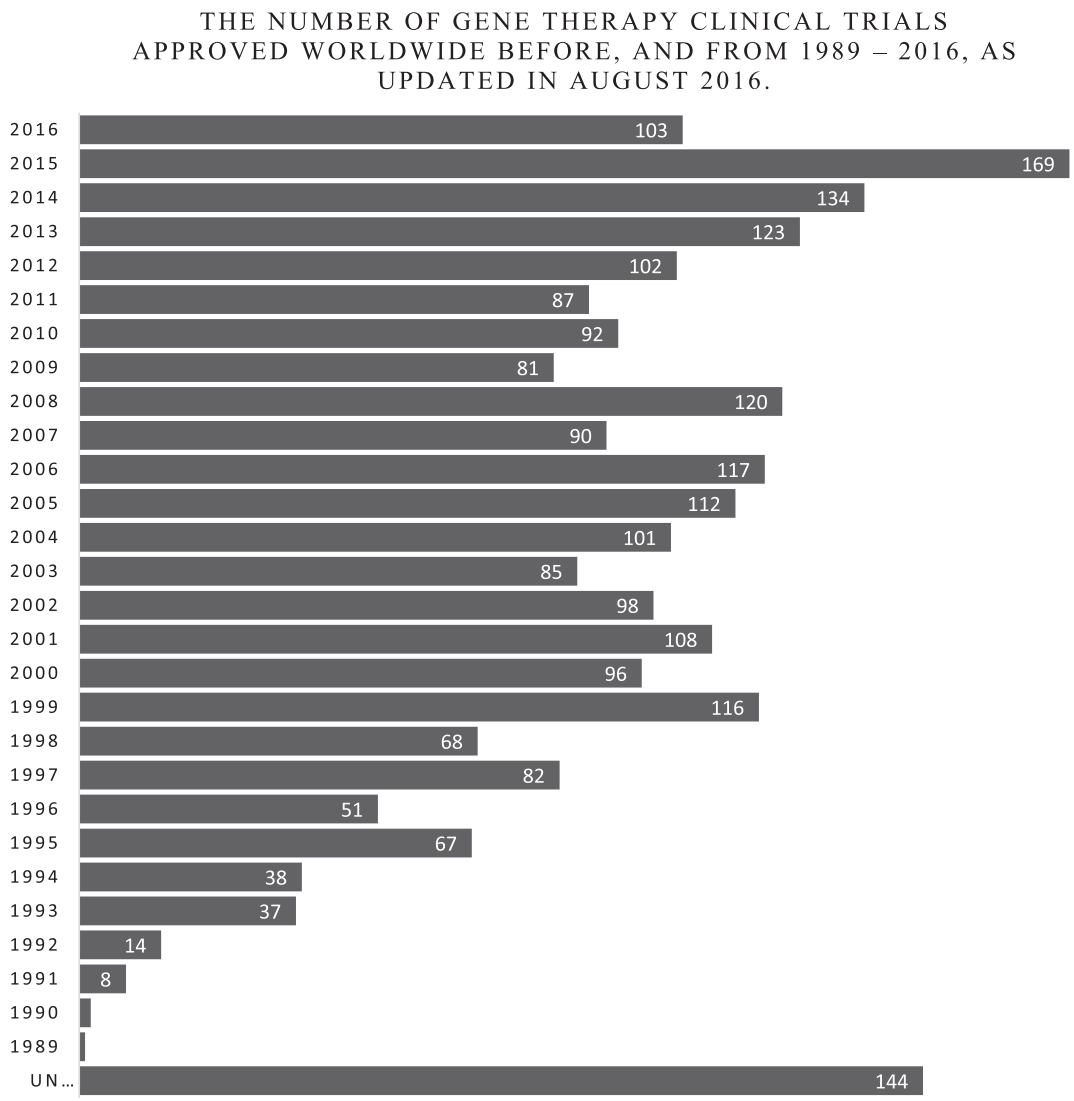

Fig. 1. The Number of Gene Therapy Clinical Trials Approved Worldwide before, and from 1989 to 2016, as updated in August 2016. Data Sourced from: (www.wiley.co.uk/genmed/clinical) on 2nd June, 2017.

Table 2
Phases of Gene Therapy Clinical Trials. Data Sourced from: (www.wiley.co.uk/genmed/clinical) on 2nd June, 2017.

Clinical Trial Phase	Number (n)	Percentage (%)
Phase I	1409	57.20
Phase I/II	500	20.30
Phase II	429	17.40
Phase II/III	24	1
Phase III	93	3.80
Phase IV	3	0.10
Single subject	5	0.20
Total	2463	100

endonucleases (initiating a double-strand DNA break (DSB) at a specific locus) by taking advantage of the functional separation of transcription factors resulting in a DNA-binding domain as well as a transcription regulatory domain, thus recruiting the DNA repair machinery to the specific site. An in-trans, homologous recombination will result in a specific genomic site integration of the endogenous sequences by providing an exogenous DNA with arms that are homologous to the sequence adjacent to the DSB, which are corrected by the non-homologous end-joining (NHEJ) repair machinery that creates mutations at the DSB site. Although the off-target genotoxicity along with cytotoxicity of the designer nucleases are a major consideration that has to some extent, delayed the translation of these experimental results to clinical practice. In perspective, one great strategy of this area of gene therapy in exploiting rationally designed single guide RNA is that it is endowed with the ability to recruit corresponding nucleases to any spot on the human genome, facilitating 'vectorization', providing site-directed integration of the therapeutic cassette into a specific target locus (e.g. AAVS1 locus, located in between exon 1 and intron 1 phosphatase 1 regulatory subunit 12C gene), minimizing dysregulation of gene expression, while still protecting the cassette from epigenetic effects [14]. Without any alterations in the transcriptional behaviour of genes adjacent to the target gene, Zinc-finger nucleases (ZFN)-mediated site-specific recombination at such site produces a sustained transgene expression [15,16]. In attempting to apply these findings in molecular therapy to treating HIV-1 infection, over 30 HIV-patients have been cared for with zinc-finger nuclease-modified T cells, in a multicentre clinical trial initiated; focusing on specific gene disruption by a zinc-finger nuclease to target the HIV-1 co-receptor CCR5 in order to protect T cells from new infection, as prophylactic for this T cells which are the main target of HIV-1 [17,18]. The mechanism involves a disruption of CCR5 locus, which results in a sustained improve-

ment in CD4 counts resulting presumably from the long-term maintenance of CCR5-modified central memory CD4 cells [19].

2.2. Immunotherapy based on chimeric antigen receptor (CAR)

Beyond genetic diseases lie an ocean of other non-genetic disease types treatable by gene therapy. Such an application is cancer immunotherapy, which is based on the genetic modification of autologous T-cells. It is effective in exterminating B-cell leukemias and lymphomas resilient to conventional therapies. The mechanism of the genetic modification of autologous T-cells is based on engineered autologous CD8+ T-cells designed to distinguish and destroy cells bearing tumor-specific antigens via a CAR. The great characteristic advantage of the CAR is that it combines the specificity of monoclonal antibody, cytotoxic and the proliferative abilities of activated CD8+ T-cell. Different generations of CAR-modified autologous CD8+ T-cells developed with variations of signaling domains have been created through *ex vivo* gene transfer using Lentiviral Vector [20–22] expressing varied efficacy. Clinical trials employing modified T cells have used CARs in correcting relapsed as well as B-cell lymphoma, refractory B-cell leukemias, acute lymphoblastic leukemia (ALL) (#NCT01044069) and chronic lymphocytic leukemia (CLL) (#NCT00466531). As opposed to the advantage of lack of cytokine release syndrome, and the rapid crowded motility of 19-28z+ T-cells to the locations of CD19+ tumor, these procedures were not without setbacks such as *p53* gene deletions, deficient genetic prognostic markers, and displayed advanced disorder as evidenced in bulky lymphadenopathy. Clinical observations (#NCT01029366, #NCT00924326, #NCT01087294) from targeting CAR T-cells (>1000-fold) containing a co-stimulatory domain from CD137 (4-1BB) in addition to the T-cell receptor ζ-chain (CTL019) with CD19 demonstrated potent non-cross-resistant activity preceding infusion in CLL [23], with persisting memory CAR+ T cells that retained anti-CD19 effector functionality. The varied long term positive feedback from these clinical trials such as reversible transient toxicities, rapid tumor eradication as well as lack of graft versus host disease has opened the door to extending these trials to further trials (#NCT01029366, #NCT01593696, #NCT01044069 and #NCT01626495) involving the more hostile B-ALL, expressing the CD19 antigen also [24–28]. The setbacks associated with some of these trials only point to the need to improve on CARs that recognize other tumor-associated antigens in B-cell lymphomas and leukemias [29]. The transfer of T-cell receptor (TCR) genes to fusion proteins into B cells or Treg presumably fosters clinical immune tolerance induction [30,31]. Likewise, CAR NK cells have been demonstrated to be cytotoxic to B-cell leukemia, as well as transducing CARs into FoxP3+ regulatory CD4+ T cells (Treg), leading to specific immunosuppression [32–34]. CAR gene transfer to T and NK cells also shows potential for generating immunity to virus [35]. Optimal co-stimulatory signaling domains or suicide gene switches are very viable options being considered for better safety strategies as well as the arduous conditioning regimens in these treatment approaches. Promisingly, CAR T-cell therapies could also be a viable option for solid tumors, human immunodeficiency and other malignancies.

2.3. Updates in Inducing Pluripotent Stem Cells

Though improved as well as more reliable protocols such as engraftable HSC leading to transplantable cell types in which there is an integration of the modification into their natural niches *in vivo* exists; where autologous transplants compatible to the recipient's immune system are hoped for [36,37], other remarkable efforts such as the use of iPSC which has the potential of expanding our pool of molecular therapies and possibly offer fascinating perspectives for the management of various disorders must be acknowledged. One very important tool with a very large reservoir of potentials in molecular therapy strategy is the Pluripotent Stem Cells (PSC), with pluripotency factors that include Klf4, Sox2, c-Myc, Oct4, which can be generated from any individual, with the greatest potential of differentiating into disease-associated cell phenotypes of three germ layers, comprising of mesoderm (e.g. cardiac cells, blood and muscle), endoderm (liver, pancreas) and ectoderm (epidermis, neurons). A lot of evidences have supported the underlying mechanism that mature cells can be 'reprogrammed' into immature PSC by nucleus transfer becoming induced Pluripotent Stem Cells (iPSC; monoclonal in nature), hence, disease specific iPSC (the differentiated mature progeny) could be designed from patients' somatic cells [38–42]. One of the viable therapeutic options to treating causative genetic anomalies in monogenetic disease-specific is the iPSC obtainable by using strategies that include non-integrating (sleeping beauty transposon) as well as integrating viral vectors (including retroviral vectors), mRNA and protein delivery. Despite all the risks associated with insertion associated transformation, a reasonable percentage of an encoded transgene using lentivirus occur in safe harbour which allows for a retained globin expression in b-thalassaemia iPSC as well as their differentiated cell lines progeny [43]. A good application of cell and gene therapy, highlighting the potential of iPSC strategies in disease modelling as well as therapeutic options is also reported in the restoration of CGD by using AAVS1 as the vector where the therapeutic cassette is integrated into the locus with the aim of restoring superoxide production in the granulocytes which are derived from the targeted iPSC obtained through homologous recombination design [44,45]. Fascinatingly, Clinical trials involving iPSC are already underway, where scientists have plans to use iPSC-derived cells to treat macular degeneration (a devastating age-related eye disease) (http://www.nature.com/news/stem-cells-cruise-toclinic-1.12511).

3. Conclusion

Several gene therapy trials have confirmed the clinical benefits of the application of molecular biology and biotechnology tools in handling diseases or disorders that have proven elusive to cure using the conventional pharmacotherapy. Interesting clinical achievements in various diseases types have been recorded over the last decade. Gene therapy has been remarkably applied to rare diseases, and in other applications such as in tumor suppressors, oncogene downregulation, vector-directed cell lysis, suicide genes, ocular, inherited immunodeficiency disorders, congenital eye diseases, hemophilia B, lipoprotein lipase (LPL) deficiency, muscle disorders and other inherited neurological disorders. There have been apparent ethical, scientific, and technological challenges militating against gene therapy, however, a lot of efforts have been made to facilitate the efficient translation of gene therapy into clinical practice. Recently and worthy to be noted is the application of modified T-cells (using TCR and CTAs), Chimeric Antigen Receptor (CAR) and other molecular biology tools in recent gene therapy. The application of gene therapy to these disease areas has redefined diseases previously classified as incurable as curable.

Conflict of Interest

None declared.

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