Exciting Avenues of Programmable bacteria for cancer therapy

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Please do write to us with your comments and feedback. Your suggestions are always appreciated.

We do hope that you will enjoy reading this issue of Biotechnology Kiosk.

Dr. Megha Agrawal and Dr. Shyamasri Biswas

*Executive Publishers and Editors*
CONTENTS

VOLUME 1, ISSUE 3          AUGUST 2019

COLUMNS

REGENERATIVE MEDICINE
The first Human Stem Cell derived Blood Brain Barrier Organ Chip – The New Age of Precision Neuromedicine

GENETIC ENGINEERING
Exciting Avenues of Programmable bacteria for cancer therapy

IMMUNOTHERAPY
Harnessing the potential to fight from within – immunotherapy advances in cancer treatment

GENE THERAPY
Exciting Advances in DNA vaccine: A New Hope to Targeted Gene Therapy for Complex Diseases

BIOTECH R&D AND INNOVATION NEWS

EDITOR’S PICKS: BIOTECHNOLOGY ADVANCES AROUND THE WORLD
Cardiovascular Biotechnology
Ophthalmic Biotechnology
Plant Biotechnology
Stem cell Biotechnology
BIOTECHNOLOGY AND PHARMA INDUSTRY ROUNDUP..........................................................38

FDA grants designation to (dapagliflozin)........................................................................38
Ethical principles for gene editing....................................................................................38
Zogenix acquires Modis Therapeutics..............................................................................38
Amgen acquiring Celgene's Otezla..................................................................................38
FDA approves Biobeat......................................................................................................38
NuVasive develops spine implants..................................................................................39
Freenome and ADC Therapeutics enter collaboration......................................................39
Pfizer investing in gene therapy.......................................................................................39
Fujifilm starts medical device headquarter.......................................................................39

ADVERTISING INFORMATION.......................................................................................41

(a) WEB BANNER AND ADVERTISEMENT RATES
(b) GENERAL AD RATES FOR THE MAGAZINE
“Precision medicine”, is the new lexicon in the field of clinical research and medicine which is being looked upon as one of the most promising and futuristic healthcare solutions. The concept of precision medicine stems from the term “personalized medicine” which has become a popular approach in the field of stem cell therapeutics and regenerative medicine. The word ‘personal’ evokes a feeling that therapies and treatments are being customized for individuals, which sounds progressive but could lead to a misinterpretation that research is meant only for the rich and the influential. However, the word ‘precision’ clearly highlights that treatments are being designed for patients on the basis of environmental, genetic and lifestyle factors which implies research shall serve mankind in larger interest. Time and money are the two major deciding factors which hold the potential of transforming a simple research idea into a life transforming technology. Same is the case with “precision medicine” which is like targeted bombing; it is accurate, precise and hits upon a specific target eliminating the root cause of the problem. Stem cell derived ‘Organ on Chip’ technology has completely revolutionized the field of regenerative medicine and many functional human organs have successfully been recreated. The latest advancement in the field is the recreation of the Blood Brain Barrier (BBB) of the human brain in the form of an organ chip which mimics the disrupted functionality associated with brain disorders. Patient specific induced pluripotent stem cells (iPSCs) were employed for the purpose and thus the research endeavor has carved a niche in the field of “precision Neuromedicine”.

Why recreate the Blood Brain Barrier (BBB)?

The Blood Brain Barrier (BBB) is the collective name given to the unique microvasculature of the CNS (central nervous system) and comprises a complex interplay between different cell types such as the endothelial cells of the blood vessels, the pericytes, neurons, astrocytes and the microglia. The BBB is a structurally and physiologically distinct unit of the brain and
adds on to the complexity of the organ which continues to intrigue researchers till date. It regulates the entry of solutes, nutrients, ions, toxins and pathogens and thus plays a crucial role in the CNS homeostasis. The BBB is pivotal in maintaining proper neuronal function, prevents injury, inflammation and safeguards the delicate structures encased within. However, it serves as an obstacle in the route of therapeutics and drugs which cannot be directly delivered into the brain. A disrupted and functionally impaired BBB is a characteristic hallmark of several neurodegenerative diseases and neurological disorders. Thus recreating this complex structure outside the human body will widen the horizon of neuroscience research in health and disease. Figure 1 depicts the cellular architecture of the blood brain barrier in a normal brain as well as a tumor containing brain (1).

Figure 1: The cellular architecture of the blood brain barrier in a normal as well as a tumor brain. [Source: Advanced drug delivery reviews, 2017].

The technical knowhow underlying the Human ‘Blood-Brain Barrier Organ on Chip’.

Several attempts have been made to recapitulate the complex physiology of the human brain within a laboratory but it is evident that we need to assemble the various
structures of the brain one at a time in order to complete the big jigsaw puzzle called the ‘Human brain’. The BBB has been a lucrative target for recreation and met success earlier however, this time researchers from Ben-Gurion University of the Negev (BGU) and Cedars-Sinai Medical Center in Los Angeles have jointly replicated a patient’s BBB creating a human BBB chip. They have for the first time employed patient specific induced pluripotent stem cells (iPSCs) for the purpose, which can be used as a potent tool to develop precision medicine and design novel approaches to research upon CNS disorders (2).

Induced pluripotent stem cells (iPSCs) are the normal somatic cells of a body which can be genetically reprogrammed into functional stem cells which can then be directed to convert into any specific cell type or lineage. These somatic cells can be derived from the skin, blood or any normal healthy tissue of the body. For this study patients’ blood samples were used to obtain functional iPSCs. Further induced pluripotent stem cell (iPSC)-derived brain microvascular endothelial like cells (iBMECs), neurons and astrocytes were cultured and then planted inside ‘organ chips’ so as to mimic the physiological microenvironment and the natural mechanical forces that the cells experience in-vivo. The organ chips were procured from Emulate, Inc. in Boston, which is the commercial manufacturer of the ‘Organs-on-Chips’ technology.

To briefly describe, organ chips are micro devices made up of flexible PDMS (polydimethylsiloxane) elastomers. The chip employed for the study comprised two micro-channels of the size 131 mm and 130.2 mm that were aligned in parallel. The two channels representing the brain and blood side were further separated by a porous flexible PDMS membrane which was laminin coated and provided a porosity of 2% over a surface area of 0.171cm². The brain side was coated with laminin and the blood side with a mixture of fibronectin and collagen. These biopolymers help in replicating the structural complexity of the human BBB. In order to function like an actual human blood brain barrier the device was connected with a microfluidics system which mimics human blood flow and a Human Emulation System which recreates the exact microenvironment needed for physiological functionality (3).

**Figure 2** shows the schematic representation of the BBB organ chip showing the cellular components as well the biopolymer coated micro device (2).

Morphological, functional and physiological parameters were assessed to validate the organ chip model. Morphologically the iBMECs expressed the entire set of cellular markers specific to the vasculature of the brain and the chip exhibited trans endothelial electrical resistance (TEER) which is a physiological characteristic of the BBB (4). When the vascular lumen was perfused with blood, the neural cells were protected from plasma-induced toxicity by the micro engineered capillary wall system. The chip further demonstrated functional blood-to-brain permeability and accurately predicted the transport feasibility of drugs and pharmacologics in case of CNS disorders. Further when patient derived iPSCs were used to create the BBB chip the barrier replicated the disease specific malfunctions.
In this case iPSCs of individuals suffering from Allan-Herndon-Dudley syndrome (an unusual congenital neurological disorder) and Huntington's disease (a neurodegenerative disorder) were employed and the Chip showed the exact lack of disease specific transporters and disrupted barrier integrity as present in the patients. Thus the BBB organ chip tested positive on all molecular, structural and functional parameters and was validated as a functionally sound model which is by far the closest possible mimic of the human blood brain barrier system.

Figure 2: Schematic representation of the ‘BBB organ chip’ showing the cellular components as well the biopolymer coated micro device. The iBMECs were seeded on the blood side and primary human pericytes and astrocytes were seeded on the brain side. [Source: Cell stem cell, 2019].

The current and future prospects of the technology

The BBB chip is a first of its kind human testing model system which blurs the boundary between in vivo, in vitro and in silico approaches. It is a perfect combination of innovative stem cell science, software design, instrumentation and microfluidics technology. ‘Organ on chip’ models are promising tools that can play a pivotal role in basic research, diagnosis and treatment. The brain is one of the most complex organs of the body and so are the implications associated with it.
A major problem being that any form of CNS disorder cannot be diagnosed directly via path-lab tests as reflective markers of the brain are not present in the blood (5). Neurodegenerative diseases as well as neurological disorders spell doom, uncertainty, hefty financial strain and helplessness. Even a lay man is aware of the complications associated with brain related disorders. ‘Every Human being is unique’. 

Figure 3: A complete summary of the ‘BBB organ chip’ depicting the technical knowhow involved, the underlying scientific concept as well as the applications of the device. [Source: Cell stem cell, 2019]
this cliché probably derives its origin from biology as it highlights the fact that humans are genetically, phenotypically and environmentally diverse. Treatments are common but how a patient responds to it will depend on its genetics, immune system, environment and lifestyle. Therefore it is improbable to administer the same set of pharmacologics/drugs to every patient which might just lead to a toxic overload of drugs without any respite. Targeted therapy and precision medicine take the patients' individual parameters into account and thus enables us to customize treatments which shall be effective and devoid of deleterious side effects. **Figure 3** is the conceptual summary of the ‘BBB organ chip’ and clearly depicts the current and future applications of the technology (2).

Customizing treatments for patients is an age old tradition where the medical practitioners usually asks patients regarding allergies and dietary habits before a treatment but technology has taken this to another level altogether. The ‘BBB organ chip’ derived from patient iPSCs is a novel tool which can help in predictive diagnostics, screening the efficacy of drugs, patient specific pharmacokinetics, identification of potent molecular targets and thus focused treatment strategies. This technology has opened new exciting possibilities in the field of neuroscience research and medicine as it is cost effective, rapid, reproducible and also potentially applicable for humans. With new technological innovations and unprecedented research endeavors we are gradually heading towards an era of futuristic “precision neuromedicine” which shall completely redefine the way we currently look at and deal with the human neurological disorders and neurodegenerative diseases.

**References**


Genetic Engineering
By Navneeta Kaul, PhD
Contributing Editor

Exciting Avenues of Programmable bacteria for cancer therapy

Genetic engineering of living cells is leading a new era of medicine. Using genetic engineering, scientists have programmed bacteria as a therapeutic delivery system to destroy tumors in mice (1). So far, designing a safe, efficacious, anti-tumor response without toxicity and within a solid tumor has remained a challenge. However, recent research has demonstrated that bacteria could be programmed as an effective cancer therapy. In this editorial, we will describe recent advances made in genetic engineering of bacteria for cancer therapy.

Bacteria loaded with nanobodies:
Our immune system is capable of targeting and killing cancer cells on its own (figure 1). However, certain tumor cells are resistant to immune cells and other macrophages due to a gene called CD47 (2). Normally, this gene encodes for a protein to coat the surface of Red Blood cells (RBCs) (3). This signal is interpreted as a kind of “Don’t eat me” signal and thus evades the check by immune cells. As RBCs age, CD47 proteins are lost and are engulfed by immune systems, leading the way for new RBCs.

Mutations in cancer cells allow them to switch on the CD47 gene, rendering them to pass through immune cells and metastasize to other places, turning them to grow into tumors. A lot of efforts focused on targeting antibodies to attach to CD47 and mask the signal. However, due to large size of antibodies, tunneling through large tumors becomes a daunting task (3). Also, injection into bloodstream could cause a random dispersion to other cells, causing side effects. In a recent paper published in Nature Medicine, scientists have engineered a non-pathogenic Escherichia coli strain capable of colonizing tumors and deliver immunotherapies within the tumor (1). Researchers programmed bacteria to turn off the ‘don’t eat me’ signal on tumors and triggering an anti-tumor response. This synergistic effect of E. coli capable of lysing within the tumor, inducing local inflammation,
and blocking CD47 triggers proliferation of T-cells within the treated tumors for clearance. Further, these bacteria were programmed with the ability to produce smaller and potent antibodies against CD47, called nanobodies.

The researchers inserted a gene for nanobody turning them into nanobody factories, followed by an injection of 5 million of programmed bacteria into mice tumors (3).

![Image](image)

**Figure 1:** A typical Scanning Electron Microscopy (SEM) image of immune cells targeting tumors **[Source: Steve Gschmeissner/ Science Source].**

Additionally, the drug delivery system was set to mechanism of quorum sensing, which ensured a mass suicide for bacteria upon multiplication, releasing nanobodies (2). Moreover, the team also demonstrated that the fragments of dead bacteria could invade hidden cancer cells. These bacteria could multiply within the besieged tumor, commit suicide, deliver another round of nanobodies, and fragments (figure 2).

The nanobodies owing to their small size, were easily cleared by the body, suggesting potential to reduce the side-effects of treatment. The team also demonstrated that this therapy could also shrink tumors at distant places, which could be due to an augmented recognition by immune cells. Further proof-of-concept tests, safety, and toxicology tests in various advanced solid tumor settings were shown in mouse models (4). The success of these tests could further translate to clinical trials in patients. The ultimate goal would be to treat some forms of metastatic cancer with pills of programmed bacteria. It remains to be seen how powerful these bacteria could be in a human setting. This transformative approach could help prime a precise, efficient cancer therapy, without the side-effects of conventional drugs.
Figure 2: Histology image of bacteria multiplying within necrotic regions of lymphoma tumors (left). Programmed bacteria are undergoing waves of growth and lysing leading to immunotherapeutic release (Right). [Source: https://www.eurekalert.org/multimedia/pub/205382.php].

Genetically engineered bacteria:
Recently, multiple approaches have been developed to express reporter genes such as cytotoxic proteins, anticancer agents, and tumor-specific antigens (5). Clostridia strains including (C acetobutylicum and C beijerinckii) have demonstrated immense promise to express genes encoding bacterial enzymes (cytosine deaminase, nitro reductase), or tumor necrosis factor (TNF-α) to trigger antitumor effects. Clinical trials with programmed S typhimurium and Clostridium novyi-NT expressing HlyE or Stx2 or rec A (crucial protein for DNA repair and maintenance) have demonstrated their ability to activate the host cytokines including Interleukin-2 (IL-2), IL-4, IL-8, CC chemokine 21, leading to effective tumor reduction (6)

Bacteria as immunotherapeutic agents:
Cancer immunotherapy usually involves targeting specific immune responses within the host system to target cancer cells (5). Bacterial infections caused by C novyi could lead to the release of heat shock proteins (Hsp70), and pathogen-associated molecular patterns (PAMPs). While Hsp70 drives dendritic cell maturation, PAMPs activate interferon gamma (IFN-γ) and Th-1dependant cell-mediated response, driven by CD8+ cells (5). Research shows that CD8+ cells derived from C novyi NT-treated mice stimulates acquired immunity in a tumor-specific model. In another interesting approach, scientists took advantage of a type-3 secretion system (T3SS) of S typhimurium to infect tumor cells. However, more research is underway to fully understand its mechanism of action. Similarly, some bacterial compounds including CpG oligonucleotides could be used for dendritic cell stimulation and complete regression of B16F10 melanoma tumors (5).
**Bacterial toxins or enzymes as anti-cancer agents:**

Various bacterial toxins have the capability to suppress the immune response of the infected host. Researchers are investigating the potential of these toxins as an anti-cancer therapy, as they are potent inhibitors of antibodies and cytokines (7). Among these toxins are highly specific enzymes with the ability to alter their substrates in the cytosol, change cellular function, morphology, and even kill the host cells. Cytolysin A (Cly A), is a bacterial toxin that induces caspase-mediated cell-death by making pores in eukaryotic cell membranes (5). Studies have demonstrated that by treating mice with *S. typhimurium* or *E. coli* strains expressing the Cly A toxin inhibited tumor growth. Similarly, TNF-related apoptosis-inducing ligands (TDAI-I), FAS ligand (FAS -I), and TNF-α selectively lead to programmed cell death via death receptor pathways.

Zheng et al developed a biotic/abiotic hybrid system, in which they combined carbon nitride (C₃N₄) with an *E. coli* strain to produce nitric oxide (NO) (8). C₃N₄ loaded bacteria were accumulated within the tumor, with an enhanced ability (80% inhibition of tumor growth) to destroy cancer cells. Cyclomodulins, a class of bacterial toxins, that could inhibit or activate the eukaryotic cell cycles are also being intensely researched upon as anti-cancer agent including CNFs and CDTs. These toxins could be associated with reduced side-effects compared to conventional anti-tumor activity (5).

**Conclusions**

Despite a lot of progress in the field, there are still many challenges for widespread use of programmable bacteria as anti-cancer agents. Bacterial toxicity, DNA instability, limited targeting efficiency, choice of safe bacterial strains, and testing combination with other therapies are some of the current obstacles. However, with the intense research efforts, further advances in synthetic biology combined with effective genetic engineering, unique biochemical properties of bacteria could be developed for a whole host of advanced applications for treating cancer.

**References**


Harnessing the potential to fight from within – immunotherapy advances in cancer treatment

Strategies to treat cancer have largely involved surgical removal of the tumor, broad chemotherapies or radiotherapies that exterminate both tumor and non-tumor cells, and targeted therapies to specifically block functional pathways to eliminate tumor growth. However, despite the type of treatment, cancers often reach a refractory period after prolonged treatment, when the patients fail to respond, leading to disease recurrence. A likely cause for this recurrence is the escape of the tumor cells from immune surveillance, a process that allows the tumors to evade attack by the immune cells and trigger their constant growth. The positive and negative regulators of both innate and adaptive immune cells work in concert through multiple interactions to recognize and eradicate tumor cells. Thus, immunotherapy offers hope in cancer treatment. To this end, biotechnologists together with immunologists are working on challenging medical problems to innovate new therapeutic pathways for cancer therapy. In this editorial, we will discuss recent advances in immunotherapy that are mostly based on countering the pro-tumor properties of the negative regulators of the immune system, to enhance their anti-tumor response.

The role of T cells in immune response

The tumor microenvironment (TME) usually is infiltrated by numerous immune cells, of which the CD8+ cytotoxic T-lymphocytes (Tc) are the mainstay. The Tc cells recognize tumor specific antigens presented through antigen presenting cells (APC) and with the aid of CD4+ helper T-lymphocytes (Th) cells, kill malignant cells. However, a subgroup of the Th cells called regulatory T-lymphocytes (Treg) has anti-inflammatory and immune-suppressive properties that prevent the activation and cytotoxicity of Tc. Tumor specific T cells express certain checkpoint proteins to regulate their function in immune response. In a normal anti-cancer response, checkpoint proteins on the T cells bind to receptors on tumor cells and prevent T cell effector function, thereby preventing T cell exhaustion, a process which facilitates the retention of antigen-specific T cells in the
repertoire under chronic stimulation. This process in turn is used by the tumor cells in their own favor to suppress the immune response and trigger their continuous growth. These checkpoint proteins have thus evolved as targets for immunotherapy since blocking them using checkpoint inhibitors (CPI) can release the brake and activate T cell immune response.

**Types of immunotherapies**

Current immunotherapy strategies can be categorized into two major groups – active and passive, depending on how the method reengages the patients’ immune system to enhance anti-tumor activities. The active arm primarily includes cancer vaccines, immunomodulatory antibodies against immune checkpoint proteins and immunostimulatory cytokines to induce the hosts’ immune system.

The passive arm also known as adoptive cell transfer (ACT) is a type of cell-based therapy where tumor infiltrating lymphocytes (TIL) are isolated from the patient, engineered to express a tumor specific neoantigen to enhance their immune potential, followed by lympho depleting chemotherapy before reinfusion of the engineered immune cells back into the patient. Particularly, T cells are engineered to produce an anti-CD19 chimeric antigen T-cell receptor known as CAR-T cells (1).

**Cancer vaccines**

Vaccination was earlier considered to play a protective role only against infectious diseases, and was not considered as an effective strategy in cancer therapy, until sipuleucel-T (Provenge) was first approved by the FDA in 2010 to treat patients with metastatic prostate cancer. Since then, therapeutic cancer vaccines have been developed using antigens from cancer cells, nucleic acids that can generate tumor associated antigens or weakened cancer cells carrying a specific antigen. Oncolytic viruses can also be engineered to act as cancer vaccines and the first genetically modified virus that received FDA approval in 2015 to treat melanoma was Imlygic (talimogene laherparepvec). Imlygic is a genetically modified oncolytic virus designed to replicate within tumors and produce an immunostimulatory protein called granulocyte-macrophage colony-stimulating factor (GM-CSF). Two main reasons prevented Imlygic’s success, first was its intra-tumoral mode of administration that limited it to melanoma and failed to benefit visceral lesions, second was the competition that it faced from CPIs. Thus recent trials have explored combinations of this vaccine with CPIs in two melanoma trials. The objective tumor response rate was almost doubled in the combination arm compared to the CPI alone arm, in a Phase Ib/II trial where Imlygic was combined with the anti-CTLA-4 antibody ipilimumab. It will be interesting to see the results of the ongoing KEYNOTE-034 Phase III trial in patients with unresectable melanoma, which is evaluating the efficacy of Imlygic in combination with the PD-1 inhibitor pembrolizumab. Data from a recent Phase 1b trial where a neoantigen cancer vaccine candidate NEO-PV-01 was combined with the checkpoint PD-1 inhibitor nivolumab in advanced or metastatic melanoma, smoking-associated non-small cell lung cancer (NSCLC) and bladder cancer, showed
consistent prolongation of progression free survival (PFS) in patients from all three cancer types (2).

**Checkpoint inhibitors**

Although our immune system confers a surveillance mechanism to prevent invasion by external agents, maintaining the immune homeostasis is critical to prevent immune response towards self-proteins. Immune checkpoint proteins make sure this balance is maintained while keeping tumor antigens and infectious agents at bay. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) are two such checkpoint proteins that bind to their receptors B7 (CD80/CD86) and PD-L1 respectively, expressed on tumor or APC cells, and prevent the T cells from killing cancer cells (figure 1).

![Figure 1: Mechanisms of action of anti-PD-1 /PD-L1/CTLA-4 antibodies in the clinic using monoclonal antibodies as checkpoint inhibitors. [Source: ESMO Open. 2017]](image)

Multiple inhibitors which are mostly monoclonal antibodies against PD-1, its binding partner PD-L1 and CTLA-4 are already in the clinic showing great promise in certain cancer types. Ipilimumab (Yervoy), a monoclonal antibody against CTLA-4 was the first immune checkpoint inhibitor approved by the FDA in 2011, for treating unresectable metastatic melanoma. Despite increased response rates (overall survival and recurrence free survival) the use of ipilimumab was restricted, due to the serious immune related adverse events inflicted by this drug. The first anti-PD-1 inhibitor to receive FDA approval for the treatment of advanced melanoma was pembrolizumab (Keytruda) in September of 2014, shortly followed by nivolumab (Opdivo) in December 2014. Both these drugs were later approved for other cancers including advanced nonsmall cell lung cancer (NSCLC), metastatic head and neck squamous cell carcinoma.
(HNSCC), classic Hodgkin lymphoma, with metastatic gastric and cervical cancers where tumors express PD-L1, sorafenib resistant hepatocellular carcinoma, first line therapy for advanced renal cell carcinoma, metastatic squamous cell carcinoma of the esophagus with varying indications. Atezolizumab (Tecentriq) the first anti-PD-L1 inhibitor was approved by the FDA initially in 2016, to treat locally advanced or metastatic urothelial carcinoma following platinum based chemotherapy. Later atezolizumab has also been approved to treat patients with metastatic NSCLC and for the first-line treatment of patients with metastatic non-squamous NSCLC in combination with bevacizumab, paclitaxel, and carboplatin. A 26% overall response rate (ORR) was observed using pembrolizumab in a Phase I trial in 173 ipilimumab refractory melanoma patients. In another trial ipilimumab treatment resulted in 60% of patients showing severe grade 3 or 4 adverse immune reactions. In comparison, a study evaluating nivolumab across multiple cancer types in 296 patients, showed a 28% ORR in melanoma with only 14% grade 3 or 4 toxicities across all patients. These and other results have shown that anti-PD-1 based treatments are more favorable with regards to overall efficacy and reduced toxicity as compared to anti-CTLA-4 based treatments. This difference can be attributed to the varied distribution of these surface proteins, since PD-1 or PD-L1 are expressed on mature T cells and tumor or APC cells, whereas CTLA-4 is widely expressed on all T cells, leading to higher systemic toxicity beyond the TME (3).

With the development of these immune therapies, the fact that immunotherapy can augment the efficacy of chemotherapy, radiotherapy or even targeted therapies (eg: kinase inhibitors, hormone therapies, signal transduction inhibitors, gene expression modulators, apoptosis inducers, angiogenesis inhibitors) is now well recognized. Currently there are about 25 approved CPIs in the clinic and several 100 in preclinical and early phase clinical trials. Thus, it is not surprising that multiple combinatorial regimens using immune checkpoint inhibitors with other traditional therapies have been tested in the clinic, and multiple ongoing trials are based on these combinatorial approaches. Recently, several phase III trials have demonstrated the efficacy of combining PD-1 / PD-L1 inhibitors with chemotherapy in small cell lung cancer (SCLC), NSCLC, HNSCC and breast cancer. The synergistic potential demonstrated by combining CPIs with radiotherapy in preclinical studies have resulted in several trials evaluating these combinations in the clinic, however, with mixed results. A notable trial using this regime was a randomized phase III PACIFIC trial investigating the addition of durvalumab (anti-PD-L1) to platinum-based chemo radiotherapy in locally advanced (stage III) NSCLC, where the combination arm showed a remarkable increase in both progression-free survival (PFS) and overall survival (OS). In this particular trial the timely administration of durvalumab (14 days vs later) seemed to be critical for improving the OS in patients (1). In NSCLC, 6 trials have assessed the benefit of combining platinum based chemotherapy with anti-PD-1 therapy, and all of these showed variable, but significant, enhancement of PFS which also positively
correlated to the level of PD-L1 expression, and 4 of these trials showed improvement in OS. Based on several randomized trials, the frontline immunotherapy strategy is monotherapy in high-PD-L1, dual CPI in high-tumor mutational burden (TMB) or anti-PD-(L)1 in combination with chemotherapy in all-comers NSCLC in absence of ALK or EGFR addiction.

Despite the concern of associated immune toxicities, several preclinical studies have demonstrated the synergistic effects of combining two different checkpoint inhibitors, as manifested by increase in TIL, decrease in Treg and retraction of tumor, supporting the rationale for combining these inhibitors in the clinic. An early melanoma trial evaluating the combination of nivolumab with ipilimumab vs ipilimumab alone showed impressive ORR in the combination arm (40%) vs the monotherapy arm (20%), albeit with higher toxicities in the combination arm (53% vs 18%). Another dose-escalation study using the same drug combination in melanoma also showed prolonged progression free survival (PFS) with remarkably high ORR in the combination arm (63% vs 11%), although at the expense of higher toxicities. However, the immune related toxicities in both these trials were managed with drugs, and long term follow up revealed a longer OS in the combination arm. A subsequent well powered Phase III trial (Checkmate 067) in previously untreated melanoma also showed enhanced OS in the combination arm (58%) vs nivolumab alone (52%) and ipilimumab alone (34%); and enhanced complete response (CR) in the combination arm (11.5%) vs nivolumab alone (8.9%) and ipilimumab alone (2.2%). These and several other trials, some using pembrolizumab instead of nivolumab, all demonstrate the benefit of combining PD-1 and CTLA-4 inhibitors in melanoma treatment. The CPI combination Phase III trials in melanoma mostly showed benefit in patients with PD-L1 negative tumors (3). Early results from the CheckMate 142 Study, using the combination of nivolumab and ipilimumab in the treatment of patients with deficient DNA mismatch repair (dMMR)/high microsatellite instability (MSI-H) metastatic colorectal cancer (mCRC) showed 54.8% ORR and 78.6% disease control rate (DCR), with a manageable safety profile (1). A recent study that estimated the percentage of cancer patients who will respond to immunotherapy, reports the increase in response to checkpoint inhibitors from 0.14% in 2011 to 12.46% in 2018, showing 88% increase in response within 7 years which may be considered as a huge success. However, the efficacy of checkpoint inhibitors is still limited to certain types of cancer and better selection strategies by identifying appropriate response predictive biomarkers is necessary to expand the benefits to other cancer types.

**CAR-T cell therapy**

Currently approved CAR-T cells use autologous T-cells derived from the patient to construct a chimeric antigen receptor (CAR) consisting of a single-chain variable fragment (scFV) antigen-recognition domain, a CD3-derived T-cell activation domain, and a costimulatory domain (CD28, 4-1BB or both) (figure 2).
Figure 2: CAR T-cell products with current FDA approval use a tumor-targeting domain (derived from a mouse antibody against the human B-cell tumor antigen CD19), and signaling domains derived from human immune activating receptors (CD28 or 4-1BB) [Source: iStock/Getty Images Plus].

Tisagenlecleucel was the first CAR T-cell therapy to receive FDA approval in August 2017, for the treatment of children and young adults with B-cell acute lymphoblastic leukemia (B-ALL). This approval was based on the ELIANA trial conducted on 75 patients, that reported a strikingly high complete remission rate (CRR) of 60% and overall response rate of 81%. Most importantly, these responses were sustainable as evidenced by 80% relapse free survival (RFS) rate over 6-months, however, the treatment-related toxicity was very high, with 73% patients suffering from Grade 3-4 toxicities. Closely after this approval, in October of 2017, the second CAR-T therapy axicabtagene ciloleucel was approved for refractory aggressive lymphoma, based on results from a Phase II multicenter ZUMA-1 trial. This trial again reported a very high ORR of 83% and a CR of 58%, but was associated with a high incidence of neurotoxicity (32%). Tisagenlecleucel was approved for a second time by the FDA in May 2018, for relapsed and refractory lymphoma based on the JULIET trial in diffuse large B-cell lymphoma (DLBCL) patients, which reported 40% CR with toxicity similar to the B-ALL trial. The next in line, CAR-T therapy based on similar principles which is showing very promising response in DLBCL, with fewer toxicities in lisocabtagene maraleucel. Despite the
approval and remarkably high efficacy of CAR-T therapies, the toxicities inflicted due to cytokine release syndrome (CRS) and neuro toxicities, usually ranging between 10-50% are extremely concerning. CRS is usually manifested in fever, hypoxia and ultimately may lead to organ failure. Neurotoxicity is characterized by various neurologic symptoms, including delirium and seizures. Concurrent trials with an effort to reduce these toxicities by using IL-6 blockers or monoclonal antibodies against IL-6 along with the CAR-T therapy are underway, but they are yet to show significant reduction of toxicity. The CAR-T cells recently entering clinical trials are designed to constitutively express both the costimulatory molecules CD28 and 4-1BB ligand to enhance T cell activation and cytotoxicity. Furthermore, like resistance to most drugs, the failure of CAR-T cell therapy is partly attributed to the loss of the CD-19 receptor. To counter this, CAR-T cells are also designed to express CD-22 or to express bispecific CD-19/CD-22. A study evaluating the efficacy of CD-22 CAR in patients who had previously relapsed on CD-19 CAR, reported an impressive 73% CR rate. Currently several trials are investigating the potential of bispecific CARs, and a few of these show encouraging response data, with reduced toxicities. Though still in its infancy, the efficacy of CAR-T cell therapy as demonstrated by data from multiple trials seems to hold promise for the future, particularly in blood cancers (4).

**Need for predictive biomarkers**

Despite the advances in immunotherapy in recent years, the number of patients who actually benefit from immunomodulatory treatments are still limited, due to lack of response predictive biomarkers that can tailor specific therapies aligned to the patients’ needs. The identification of appropriate biomarkers can lead to optimization of benefits and help reduce unwanted toxicities, increase response rates and minimize costs.

Biomarkers explored in immunotherapy can be categorized into different groups such as serum proteins, tumor-specific receptor expression patterns, factors in the TME, circulating tumor cells, host genomic factors and tumor mutational burden.

High levels of interleukin-6 (IL-6) and C-reactive protein (CRP) in the serum have been reported to negatively correlate to interleukin-2 (IL-2) therapy response, in RCC and melanoma. Similarly, higher levels of soluble VEGF and CRP have been shown to correlate with lower clinical response (OS) in melanoma treated with ipilimumab. Elevated serum lactate dehydrogenase (LDH) level was also a negative predictor of benefit from ipilimumab, as analyzed from 3 separate trials. Cells in the peripheral blood, such as T cells, NK cells, dendritic cells, macrophages, and tumor cells, have been evaluated as predictive biomarkers in multiple clinical trials. Myeloid derived suppressor cells (MDSC), an immune cell population, was found to negatively correlate to the clinical benefit from ipilimumab. Some studies claimed that there is a positive correlation between increase in absolute lymphocyte count (ALC) and OR, but results from several studies put together show that irrespective of the treatment outcome there is always an increase in ALC.
The preponderance of certain cell types in the TME have also been evaluated as potential biomarkers. The reason of variations in the T cell populations residing within the TME are not clearly understood, but the types of cells present dictate whether the TME is inflammatory (increased effector T cells) or immunosuppressive (decreased effector T cells). Regardless of T cell type, the cells in an inflamed environment usually express high levels of T-cell checkpoints, such as PD-L1, B7H4, Tim-3, Lag-3 that can disable tumor-infiltrating effector cells. Furthermore, beta-catenin expression and intracellular hypoxia or release of soluble factors like IL-10 and transforming growth factor-beta (TGF-β) may result in inhibition of effector T cells. Melanomas with high Tc content, were found to be more likely to be associated with high PD-L1 expression, and resulted in improved prognosis. Similarly in NSCLC, increase in tumor infiltrating Tc and Th cells was a predictor of favorable response (5). PD-L1 expression on the tumor cells, has been the most sought after cell surface receptor that has been evaluated in multiple clinical trials as an independent predictive biomarker for favorable response. Though several studies reported a positive correlation between clinical response and PD-L1 expression on tumor cells, this is not always found to be true. This discrepancy potentially arises from the dynamic nature and heterogeneous expression of PD-L1 in patients. The FDA approved immunohistochemistry (IHC)-mediated detection of PD-L1 expression on tumors and mandated it as a prerequisite to treatment with anti-PD-1/PD-L1 inhibitors in several cancers. However, it has been found that PD-L1 tumor expression is not a reliable biomarker to determine patients who will respond. Moreover, one should not confuse between the tumors PD-L1 expression with soluble PD-L1 (sPD-L1) found in peripheral blood. Data from a study in pancreatic cancer patients suggest that sPD-1 and sPD-L1 are indicators of systemic inflammation and independent from tumoral PD-L1 expression. Another approved biomarker for predicting PD-1 inhibitor response is the presence of microsatellite instability (MSI) or deficient mismatch repair (dMMR), and several trials showed that the presence of MSI/dMMR increased tumor mutational burden (TMB) and subsequent response to PD-/PD-L1 inhibition. However, the presence of MSI/dMMR was not always associated with TMB and high TMB was itself used independently as a predictive biomarker. In the current scenario, further studies are necessary to ascertain these biomarkers as definite predictors of response (5).

Conclusions

The advancement of immunotherapy is inevitable with numerous preclinical studies and clinical trials geared to take cancer treatment to a new paradigm through immune modulation. With the discovery of better predictive biomarkers and implementation of the knowledge gained by consolidating data obtained from ongoing studies, immunotherapy will reach new heights in the near future. The CPIs will most likely expand to other cancer types and will end up gaining importance in other rational combinations. Whether the CAR-T cell therapies can be extended to solid malignancies is yet to be witnessed.
Author’s Biography

Dr. Paromita Raha is a Biotechnologist who currently leads product development and food science research at a Food technology startup, Lecker Labs, manufacturer of the world’s first automatic yogurt maker. She received her doctorate in Biotechnology from the Indian Institute of Chemical Biology in India by winning a competitive national fellowship. Later she pursued her postdoctoral research at the University of California at San Francisco in translational oncology. She has several years of research experience in preclinical oncology and she has worked as a Scientist in the drug discovery team in a pharmaceutical company. She has developed novel drug combinations in breast and lung cancer drug-resistant models, which have successfully translated to the clinic. Dr. Raha has authored multiple peer-reviewed scientific research papers in structural biology, preclinical oncology, and clinical research, and an invited book chapter in Medical Epigenetics. To remain abreast of current research, she freelances as an expert scientific reviewer and as a scientific editor. She also volunteers for outreach activities, particularly helping women and girls to pursue a career in STEM and has served as a Board of Director in a non-profit organization.

References

Gene Therapy
By Progga Sen, PhD
Contributing Editor

Exciting Advances in DNA vaccine: A New Hope to Targeted Gene Therapy for Complex Diseases

Gene therapy is the technique of using human genes to treat diseases, mostly by replacing or correcting defective genes. There are two different types of gene therapy: somatic and germline. DNA vaccines fall in the first category of gene therapy medium and is regarded as the third generation of vaccines following after the first generation (live attenuated or killed pathogens) and the second generation (recombinant vaccines) (1, 2). Edward Jenner’s ground-breaking discovery of cowpox vaccine in 1798 laid the solid foundation of the vaccine immunology field. Initially started with injecting a small amount of the antigen directly in the patient, antigen type, form, and delivery have evolved immensely. This latest and sought-after methodology involves a plasmid encoding the DNA of the antigen to be given to the patients. The various advantages with this technique contribute to a promising ground for further development in this particular field of immunology. There are various advantages of DNA vaccines that put these immunotherapeutic agents at the forefront of treatment for infectious diseases, HPV and associated disorders, autoimmune diseases, and cancer. DNA is highly stable and has therefore, a long shelf-life. Being biocompatible, easy to prepare and scale-up, DNA vaccines are a popular avenue of research for developing plausible treatment strategies. In addition, they can be administered repeatedly without worrying about side-effects.

The vaccines can be administered as naked DNA or can be encapsulated in carrier vesicles or molecules. The latter helps in protecting the DNA from digestion by DNAses and can increase immunogenicity in the host. The major routes of delivery for DNA vaccines include intradermal (ID), intramuscular (IM), mucosal, and subcutaneous injections for topical applications; they can be introduced systemically via pulmonary and oral routes (figure 1) (3, 4, 5).
Figure 1. Routes for DNA vaccine administration. DNA vaccines can be introduced into the body either by systemic applications or topical techniques. The vaccine, either in attenuated bacteria or other carriers, can be administered orally, via nebulizer (pulmonary route) or intravenous injections to ensure maximize APC transfection. Topical administration leads to the transfection of the DCs present within the human skin that has the highest distribution of DCs (200-1000 per sq mm). [Source: Int. J. Mol. Sci., 2018]

The antigen-encoded plasmids have to migrate into the nucleus of the cells- antigen presenting cells (APCs), myocytes, keratinocytes- residing near the application site. Successful transcription and translation of the plasmid DNA are followed by antigen presentation via the major histocompatibility complex I (MHC I) and/or MHC II. Eventually, the cascade of immune system activation culminates with activated CD8^+ and CD4^+ cells. B cells can get activated too, leading to specific antibody generation (figure 2). There are several factors that reinforce the importance of DNA vaccines in treating various infectious diseases as well as cancer. The three broad factors that contribute to the successful delivery and efficacy of the DNA vaccines are: 1) Plasmid construct design, 2) Adjuvants, 3) Delivery platform (6, 7).
Figure 2. Adaptive and innate immune response is generated by DNA vaccine. After the myocytes or keratinocytes are successfully transfected by the DNA vaccine plasmid, the cells express the antigen and release it via exosomes. Immature dendritic cells (iDCs) endocytose, which then present the antigen via MHC!! Complex CD4+ T cells in draining lymph nodes. Direct transfection of the APCs results in simultaneous presentation of the antigen via the MHCI and MHCII complexes, causing CD4+ and CD8+ T cell responses. Recognition of the antigen by B cells can result in humoral response by specific antibody generation. Furthermore, the intrinsic plasmid DNA can elicit innate immune response because of recognition by cytoplasmic sensors. [Source: Journal of Experimental and Clinical Cancer Research, 2019]

Plasmid construct design

The plasmid construct design plays a significant role in governing the efficacy of the DNA vaccine, and the features that are crucial for its function are discussed in brief (6). DNA vaccines are closed circular bacterial plasmids with the antigen-encoding DNA, placed under a strong promoter (figure 3). Mostly, viral promoters such as the SV40 (simian virus 40) promoter or the CMV (cytomegalovirus) promoter are used, though the latter is the first choice, given its strong expression. However, researchers are working on eukaryotic or eukaryotic-viral hybrid promoters for highly specific immune cell targeting. Ongoing works are studying cell-type specific promoters like those of the
dendritic cells, the predominant APC population in the skin. Various gene-specific promoters have been tested in mice that presented a wide variation in their abilities to activate the immune system, such as the CD11c, dectin-2, and fascin-1. Though they target a subpopulation of the entire immune cell repertoire, further refinement will help in simultaneous targeting of different immune cell types for generating a stronger response.

![Figure 3. Components of DNA vaccine plasmid.](image)

In addition to the essential origin of replication, a strong promoter, the encoded antigen and the poly A tail encoding stretch, efficacy of DNA vaccines are elevated by using one or more CpG elements that is immunostimulatory by itself, helps in augmenting the overall immune response in the host body. Antibiotic resistance gene though present for bacterial selection, certain safety-related issues in studies have prompted a preferential removal of the antibiotic encoding gene from the construct before administering into the host body. [Source: Vaccines and Vaccinations Open Access, 2018]

The plasmid also contains an origin of replication- required for the plasmid propagation in the bacteria. Another important aspect that governs the efficacy of the DNA vaccine is the size of the plasmid- the transfection efficiency correlates inversely with the size of the construct. Heterochromatin formation of the bacterial portions and spreading across the transgene are to blame for this observation. To address this issue, plasmids are treated with phage recombinase after their bacterial production, for removing the prokaryotic DNA regions. Certain groups have observed that the presence of certain antibiotic selectable marker genes can be a major safety issue for
the host- horizontal transmittance of antibiotic resistance genes to the enteric microorganisms, they can hinder the stability and efficacy of the DNA vaccine as well. To combat this drawback, RNA-based non-coding sequences are used as markers. These are small (< 200 bp) and can easily transmit to the eukaryotic host or bacteria. Codon optimization is a promising step for better expression and higher immune reaction in the host. Enhancers can be placed in the plasmid to increase the plasmid translocation into the nucleus and antigen expression at the same time as well. A well-known example is the SV40 enhancer region.

**Adjuvants**

DNA vaccines have gained popularity because of several advantages over traditional therapeutic strategies. Ease of magnifying the immune response is a crucial reason for the peaked interest of the scientific community in DNA vaccines. Adjuvants, derived from Latin “adjuvare”, means “help”. Adjuvants enhance the life and strength of the DNA vaccines besides preventing the antigen-dependent tolerance (1, 2, 3, 4). Adjuvants can be either encoded cytokines (in the same plasmid as the antigen or administered in separate plasmids), aluminium salt-based alum, prokaryotic sequences (CpG elements), components of the complement system, adhesion molecules, TLR ligands, and chemokines.

Cytokines are a favorable class of immunomodulatory molecules- IL2, IL-15 and IL-12 are extensively investigated on, they have been tested for their efficiency in stimulating heightened immune response against antigens (mostly tested in mice). IL-2 causes differentiation of naïve T cells to effective T cells; interestingly, IL-2 has been approved by FDA because of its established effect on melanoma and renal carcinoma. IL-15 induces NK (natural killer) cell and T cell proliferation and generates antigen-specific CD4+ and CD8+ T cell responses. IL-12, the first cytokine to be tested as an adjuvant, causes secondary expansion of T helper 1 cells and generates CD8+ T cells. IL-12, in combination with DNA vaccine, has been reported to decelerate myeloid immune suppressor cell (MDSC) expansion and cause mice survival against cervical cancer.

Similar to the cytokines and other immunostimulatory factors, several inhibitory receptors such as CTLA-4, PD-1 and LAG-3 have been tested for their efficacy in preventing tumor growth. Overall, in-depth work is being carried out to improve the efficacy of the DNA vaccines for various infectious diseases as well as cancer. The next important aspect of the drug is the delivery system.

**Delivery system**

The method of vaccine delivery plays an important role in the all-round success of the vaccine efficacy in the host (8, 9). Of all the different methods of administration of the DNA vaccines, intramuscular and intradermal routes work best. CO2-powered non-needle delivery is gaining popularity in vaccination for influenza and medication for cancer. Biojector 2000 is the FDA-cleared device that uses a small CO2 cartridge as its source of energy to administer vaccines and other medications preferably via intradermal, subcutaneous or intramuscular routes. Delivery using the Biojector generates Th2 as
well as CD8+ T cell immune responses. Electroporation is another improved vaccine delivery system and known to elicit Th1 immune cell response. The most advanced system CELLECTRA, developed by Inovio Pharmaceuticals, sends 3-4 optimized electric pulses to administer the DNA vaccine successfully via intradermal or subcutaneous routes. It has been tested clinically against cancer and infectious diseases alike. Gene gun is another technique where the DNA vaccine is coated around a gold particle and administered using helium as accelerator. This technique mostly elicits Th2 immune cell response, as tested in mice.

Optimization of the DNA vaccine can also be achieved by administering the vaccines in carriers (5). These carrier molecules protect the DNA from degradation and enhances the immunogenicity greatly. Often modification on the surface by antibodies or glycoproteins help targeting specific cell types, such as the dendritic cells. These carriers vary in size between 1-1000 nm and are hence called nano-carriers. Made up of natural or synthetic materials, the nano-carriers have been successfully tested to carry DNA-based vaccines, adjuvants and drugs. These nano-vaccines are a widely used drug category in cancer therapy. The carriers enhance vaccine functionality because of highly specific targeting to cells/organs/tissues, regulated drug release, and better degree of adsorption in the body circulatory system. Even though a promising approach to deliver DNA vaccines into the cells, these carriers have to combat a few limitations, including preventing lysosomal/endosomal degradation upon entering the cells, stability against serum factors (proteins) that may hinder cellular uptake, and should be large enough for the antigen-encoding plasmid.

**Clinical studies in cancer**

Several studies- clinical trials- have been and are using different combinations of targeted therapy for prostate, breast and cervical carcinomas, amongst others (4). Since DNA vaccine is still in its developing and evolving stages, it requires extensive optimization to be capable of handling a multifaceted disease like cancer. A glimpse of performed trials are outlined below:

Evaluating the degree of immune response and safety, these trials send out a mixed message, albeit provide hope. For cervical cancer, VGX-3100 was tested against HPV 16 and 18 E6 and E7 proteins; 50% patients responded with high efficacy and scored high in overall tolerance. Elevated activation of CD8+ T-mediated cellular and humoral responses were noted in the study. Another trial examined GX-188E, aimed for the same targets as the previous study for cervical cancer. Similar to the previous trial, this DNA vaccine was tolerated well and did not elicit adverse reactions events due to administration. In a study on relapsed prostate cancer, clinicians observed that combining DNA vaccine, encoding the PSA protein, with radiotherapy and endocrine treatment resulted in elevated antibody response and absence of adverse vaccine-associated events. Clinicians have developed and tested a personalized vaccine for multiple myeloma- the DNA encoded the antigen and an adjuvant (tetanus fragment C) that resulted in 72% patients with cell-specific immune response and a survival rate of 64% at the end of the trial.
Ongoing are a large number of clinical trials that are testing the efficacy of targeted DNA vaccines (with or without added adjuvants), encoding single or multiple antigens (polyepitope vaccines) and most of studies are focusing on breast, cervical and prostate cancers (figure 4). Most of these antigens are tumor-associated antigens (TAAs); increasingly, studies are including neoantigens as targets for onco-therapy. These neoantigens are identified after sequencing of DNA from tumor biopsy and subsequent comparison with normal tissue sequencing data. The unique mutations are used for generating the neoantigen-targeting DNA vaccines.

A promising field of gene-based therapy, DNA vaccines have certain disadvantages that need to be addressed before being considered as the sole treatment strategy for cancer, HIV, or the infectious diseases. Low immunogenicity, moderate to high toxicity of certain vaccine types, chance of genomic integration (though low), vaccine degradation by nucleases, and vaccine delivery methods are some of the major problems. In addition, tumors are heterogenous- consisting of different types of tumor cells and the associated problems for treatment arise from loss of or altered epitopes/antigens, antigen tolerance, immunosuppression (by MDSCs, TAMs, Tregs), and T cell exhaustion.

However, several research groups are investing their resources in understanding and refining this therapeutic strategy, especially its delivery method(s), and obtaining a standardized technique will surely propel the significance of DNA vaccines to new heights as a reliable solution to treat cancer, influenza, and HIV, HPV, to start with.

Conclusions
Latest advances in DNA vaccine based gene therapy offer many exciting avenues to combat complex diseases and serious medical conditions. DNA biotechnologists are playing key roles in developing and optimizing advanced vaccines that can be coupled with gene therapy for applications in a range of fields including treatment for infectious diseases, HPV and associated disorders, autoimmune diseases, and cancer. This field of research is growing and we anticipate further R&D and new discoveries happening in gene therapy in the future.

References

Biotechnology Advances around the World

Editor’s Picks

Every issue of Biotechnology Kiosk presents select latest research news picked by the executive editors on significant research breakthroughs in different areas of biotechnology around the world. The aim is to promote further R&D in all of these cutting edge areas of biotechnology. The editors have compiled and included the following innovations and breakthroughs to highlight the recent biotechnology advances.

Dr. Megha Agrawal
Executive Editor

Dr. Shyamasri Biswas
Executive Editor
Cardiovascular Biotechnology

New molecule shielding heart tissue during and after heart attack

An international collaborative team of researchers in the United States and China recently discovered a new molecule that could potentially shield important heart tissue before a heart attack. It is also shown as an important drug compound for administration after a heart attack that could preserve healthy cells.

In a paper published August 19 in the Journal of American Heart Association (J Am Heart Assoc. 2019; DOI: 10.1161/JAHA.119.012385), researchers characterized detailed molecular mode of action of molecule aCT1 in mitigating cardiac ischemia-reperfusion injury in mice. The compound Alpha Carboxyl terminus 1 (aCT1) is a 25–amino acid therapeutic peptide that incorporates the zonula occludens-1 (ZO-1), which is the binding domain of connexin 43 (Cx43). This compound is currently in phase 3 clinical testing on chronic wounds. In their paper, researchers demonstrated the potent cardio protective functions of a short 9–amino acid variant of aCT1 (aCT11) when infused either before or after ischemic injury.

The supply of oxygen that occurs by blood flow to the heart is vital for maintaining the health of heart cells. Without this supply of blood and oxygen, heart cells can die quickly and that is a fatal medical condition. In a heart attack, the flow of blood and oxygen to an isolated section of heart cells is significantly reduced that is known as hypoxic ischemic injury to the heart. In this potentially fatal medical condition, those damaged and dying heart cells try to send signals to their healthy neighboring cells and the injury becomes much bigger and spread out due to the fact that the surrounding area of dying heart tissue is not isolated or quarantined.

In this paper, researchers addressed this issue and possibility of keeping the injury highly localized to the group of damaged cells that are directly affected by the hypoxic ischemic injury, while at the same time letting the nearby heart muscle healthy cells to remain healthy and intact. Researchers demonstrated Alpha CT11 molecule to be even more effective than the original peptide in protecting hearts from ischemic injury similar to those that happen during a heart attack. Most importantly, this study showed that alphaCT11 can bring a robust injury-reducing effect, even after 20 minutes of the loss of blood flow that causes ischemic injury. This significant research on AlphaCT11 could pave the way to develop new therapeutic pathways to effectively treat heart attacks and prevent the spread of heart cells damage that occurs immediately after a heart attack.

Optic nerve is an indispensible part of entire ocular system. It carries the visual information that is conveyed from the eye to
the brain through the axons of retinal ganglion cells. It is quite difficult to regenerate optic nerves unlike other tissues because optic nerve belongs to the central nervous system. Damage to the optic nerve typically causes potentially severe loss of vision leading to partial or permanent blindness. Blindness affects millions of people in the world that can result from many factors including genetics, retinal detachment and trauma, stroke in the visual cortex, glaucoma, cataract, inflammation and infection. Finding a viable medical solution to the complex problem of blindness is enormously challenging. Ophthalmic implant technology advances such as retinal implants have been employed to combat blindness. However, the applications of retinal implants are limited due to the exclusion criteria. For example, retinitis pigmentosa which is a genetic disorder causes blindness in about ½ million people worldwide. Retinal prosthetic implants have been considered in such patients, but only a limited number of patients can qualify for prosthetic implants due to clinical reasons. Similarly, another strategy based on brain implant has been considered to combat blindness. Brain implant can stimulate the visual cortex directly. However it is a risky approach. In a different approach, scientists from EPFL in Switzerland and Scuola Superiore Sant’Anna in Italy have developed a new type of neural electrode to provide distinct visual signals to the blind by directly stimulating the optic nerve. They have developed a technology that bypasses the eyeball entirely and sends messages to the brain for the blind by directly stimulating the optic nerve with a new type of intraneural electrode called OpticSELINE. Their research is published in Nature Biomedical Engineering (Nature Biomedical Engineering, 2019; DOI: 10.1038/s41551-019-0446-8).

The approach of employing intraneural electrode is based on the idea of producing phosphenes that allows to generate distinct sensation of seeing white patterns of light without having the need to see the light directly. This also overcomes the current limitation of exclusion criteria of retinal implants due to the fact that in the intraneural electrode based approach, the pathway to the brain and the optic nerve remain intact. These intraneural electrodes provide rich visual information to the subjects and they are stable once implanted in a subject. Based on a developed algorithm, the scientists were able to decode the cortical signals. They also demonstrated with each stimulating electrode inducing a specific and unique pattern of cortical activation that suggested selective and informative nature of the employed intraneural stimulation of the optic nerve.

**Plant Biotechnology**

**Shedding new light on the plant immune system**

Plants are similar to humans and animals with respect to the complex immune systems that have evolved over millions of years. It is believed that these complex immune systems fend off invading pathogens. However, the shortcomings of the immune systems of plants are the lack of adaptive
immunity that come from antibodies. What this means is that each plant cell has to defend itself against all invading pathogens. Each plant cell consists of protein complexes encoded by disease resistance genes that fight against harmful pathogens such as fungi or bacteria. Such genes encode traits can be used to generate disease-resistant crops. However, much of these plant immune response and how they work are not known yet to plant and agriculture biotechnologists who are striving to protect food crops.

In a new research published August 23 in the journal Science (Science, 2019; DOI: 10.1126/science.aax1771), a collaborative team of biologists based in Spain and in the United States have shed new light on a crucial aspect of the plant immune response. Their research results revealed the mechanism for the first time on how plant resistance proteins trigger localized cell death. This very significant discovery in plant and agriculture biotechnology is expected to pave the way for the development of new strategies that would allow engineering of disease resistance in next-generation crops.

Focusing on the process during the plant immune response, researchers then made an important observation about the TIR domain that this domain is an enzyme that degrades a molecule called NAD+. NAD+ molecule is considered very essential for metabolism in all organisms. Researchers demonstrated that by cleaving NAD+, the plant self-destructs infected cells while leaving other cells unharmed.

This finding and uncovering of individual biochemical pathways in the plant immune response is critically important to reveal how plant systems can be favorably manipulated to protect food crops from new pathogens and generate superior quality food products for global food supplies.

**Stem Cell Biotechnology**

**Reversing aging process in brain stem cells**

It is known that the human ageing process is associated with a decline in tissue regeneration. This is due to a loss of function of adult stem cell and progenitor cell populations. This leads to the deterioration of the regenerative capacity of widespread and abundant population of multipotent brain stem cells known as oligodendrocyte progenitor cells (OPCs) that belong to the central nervous system.

A new research by a multi-disciplinary research team, based in UK published in Nature (Nature, 2019; DOI: 10.1038/s41586-019-1484-9) reveals that with the ageing brain-stiffening happens similar to stiff muscles and joints as our bodies age. This research demonstrates new ways to reverse older stem cells to a younger, healthier state after the age-related brain stiffening induced brain stem cell dysfunction occurs.

To understand the impact of age-related brain stiffening on the function of OPCs, researchers investigated young and old rat brains. This study resulted in the crucial
finding that tissue stiffness is a crucial regulator of ageing in OPCs. This also provided insights into the relation of the changes in the function of adult stem and progenitor cells with age. Researchers then investigated to determine whether it was possible to reverse the loss of function in aged OPCs. They transplanted older OPCs from aged rats into the soft, spongy brains of younger animals. In a significant and breakthrough finding, the older brain cells were observed rejuvenated that became more vigorous cells similar to the younger and healthier state.

These discoveries have far reaching implications that could open up further avenues for the development of regenerative therapies and much-needed treatments for age-related brain diseases along with new fundamental understanding of the ageing process itself.

Compiled and Edited by Dr. Megha Agrawal and Dr. Shyamasri Biswas.
Biotech and Pharma Industry Roundup

**US FDA grants designation to drug Farxiga for chronic kidney disease**

U.S. Food and Drug Administration (FDA) designated AstraZeneca’s Farxiga (dapagliflozin) medication as a treatment for patients with chronic kidney disease. U.K.-based AstraZeneca announced that the FDA awarded the designation to Farxiga to delay the progression of renal failure and prevent cardiovascular and renal death in patients with chronic kidney disease. The drug Farxiga was granted under the fast track designation and was assigned to chronic kidney disease patients with and without type-2 diabetes (Source: https://www.biospace.com/).

**Guiding ethical principles for gene editing issued by Alliance for Regenerative Medicine**

A bioethical framework for gene editing’s use in therapeutic applications was issued by Washington, DC-based ‘The Alliance for Regenerative Medicine (ARM)’. ARM is known as an international advocacy organization that represents the cell and gene therapy and broader regenerative medicine sector (Source: https://www.biospace.com/).

**Zogenix acquires startup Modis Therapeutics for $250M buyout**

Zogenix recently announced to expand its pipeline beyond lead drug Fintepla and acquire Modis. Zogenix will pay $250 million upfront to acquire Modis Therapeutics, a privately-held biotech enterprise. By acquiring Modis, Zogenix’s portfolio will include R&D activities on developing a treatment for an ultra-rare neuromuscular disorder (Source: https://www.biopharmadive.com/).

**Amgen acquiring Celgene’s Otezla in $13B deal**

Amgen announced that it will buy the psoriasis and arthritis drug Otezla for $13.4 billion, acquiring from Celgene. This deal will be an on-market complement to its aging top-seller Enbrel. As a result of the deal, Celgene employees between 800 and 900 currently working on Otezla will join Amgen [Source: https://www.biopharmadive.com/].

**US FDA approves ‘cuffless’ blood pressure tracking smartwatch from Biobeat**

The Tel Aviv-based Biobeat has received approval from the US FDA for its smartwatch-based patient monitoring device. This technology is designed to track a user’s blood pressure and other vital signs and does not require traditional inflated cuff. The working principal of the device is based on optical sensors that employ light to noninvasively
measure changes in the skin. This also includes pulse rate and oxygenation comparable to other devices. The company envisions for long-term use of this technology in hospitals, clinics and the home. The advantageous part is that the patch also includes a single-lead ECG. This FDA approval opens a range of opportunities for the company to promote and sell the tracking smartwatch for remote monitoring of vital signs of patients in the U.S. market (Source: https://www.fiercebiotech.com/).

**NuVasive develops porous titanium spine implants**

NuVasive has recently announced launching of its latest spine implant. This spine implant technology helps expanding its porous titanium portfolio to all transforaminal lumbar interbody fusion surgeries (TLIF) procedures. The addition of this latest implant technology is anticipated to increase the company’s footprint among interbody fusion devices, part of what it estimates as a $1.2 billion market (Source: https://www.fiercebiotech.com/).

**The US biotech company Freenome and the Switzerland based ADC Therapeutics enter biomarker collaboration**

In a recent announcement, the US biotech company Freenome will use its multiomics platform to develop response biomarkers to ADC Therapeutics’ Phase II antibody drug conjugate (ADC) ADCT-402. Under this biomarker development collaboration, ADC Therapeutics SA (Lausanne) will employ Freenome’s already established multiomics platform. The goal of the collaboration is to identify biomarkers that correlate with clinical response to ADCT-402 (loncastuximab tesirine), an antibody-pyrrolobenzodiazepine (PBD) conjugate targeting CD19 expressed on human B cells in order to eliminate B cell malignancies (Source: https://european-biotechnology.com/).

**Pfizer investing additional $500M to increase manufacturing capabilities in gene therapy**

Pfizer recently announced for an additional $500 million investment at its newly completed gene therapy plant on its 230-acre campus in Sanford, North Carolina in the US to increase manufacturing capability. This investment will add 300 more jobs in biotech sector. This expansion by the pharmaceutical company is followed by its initial expansion at its Lee County vaccine-manufacturing plant with a $100 million investment in gene therapy manufacturing that added 40 jobs that has grown now a 650-person workforce (Source: https://businessfacilities.com/).

**Fujifilm starts a single location medical device groups headquater in Massachusetts**

The FUJIFILM Medical Systems U.S.A., Inc. recently announced moving its headquarters in a single location in Lexington, Massachusetts that will mark the first time Fujifilm’s various healthcare groups—computed tomography, digital radiography, women’s health, endoscopy, minimally invasive surgery, and medical IT to be assembled and work from a single location in the United States. Their aim of placing the entire healthcare resources under one roof
was designed based on a strategic move to foster close collaboration across various business units in order to improve the customer experience (Source: https://businessfacilities.com/).
Advertising rates for the magazine:

Top Banner...................................... 728 pixels wide x 90 pixels high (includes URL link)

Medium Banner................................. 400 pixels x 90 pixels (includes URL link)

Please supply animated banners as gifs. Static banners may be supplied as gifs, PNGs or JPEGs. All banner files, static or animated, should be kept to 75k or below.

Top Page Banner Position: $600 per month

Middle Page Banner Position: $350 per month

Side and Bottom Page Banner Position: $250 per month

General AD Rates for the Magazine

<table>
<thead>
<tr>
<th>Per month rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Page</td>
</tr>
<tr>
<td>Half Page</td>
</tr>
<tr>
<td>1/4 th Page</td>
</tr>
</tbody>
</table>

For all production related questions or sending your ads, please e-mail or call our production department: E-mail: sales@biotechkiosk.com; Phone: 386-518-9411