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Theme: Chimeric Antigen Receptor T Cell: An Advanced Cell Therapy for Cancer

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Article 1:

Chimeric Antigen receptor T-cell Therapy for Cancer

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ABSTRACT

Chimeric antigen receptor (CAR)-T cell therapy is a revolutionary new pillar in cancer treatment. Although treatment with CAR-T cells has produced remarkable clinical responses with certain subsets of B cell leukemia or lymphoma, many challenges limit the therapeutic efficacy of CAR-T cells in solid tumors and hematological malignancies. Barriers to effective CAR-T cell therapy include severe life-threatening toxicities, modest anti-tumor activity, antigen escape, restricted trafficking, and limited tumor infiltration. In addition, the host and tumor microenvironment interactions with CAR-T cells critically alter CAR-T cell function. Furthermore, a complex workforce is required to develop and implement these treatments. In order to overcome these significant challenges, innovative strategies and approaches to engineer more powerful CAR-T cells with improved anti-tumor activity and decreased toxicity are necessary. In this review, we will discuss recent innovations in CAR-T cell engineering to improve clinical efficacy in both hematological malignancy and solid tumors and strategies to overcome limitations of CAR-T cell therapy in both hematological malignancy and solid tumors.

Introduction

Chimeric antigen receptor (CAR)-T cell therapy has been revolutionary as it has produced remarkably effective and durable clinical responses. CARs are engineered synthetic receptors that function to redirect lymphocytes, most commonly T cells, to recognise and eliminate cells expressing a specific target antigen. CAR binding to target antigens expressed on the cell surface is independent from the MHC receptor resulting in vigorous T cell activation and powerful anti-tumor responses. The unprecedented success of anti-CD19 CAR-T cell therapy against B cell malignancies resulted in its approval by the US Food and Drug Administration (FDA) in 2017. However, there are major limitations to CAR-T cell therapy that still must be addressed including life-threatening CAR-T cell-associated toxicities, limited efficacy against solid tumors, inhibition and resistance in B cell malignancies, antigen escape, limited persistence, poor trafficking and tumor infiltration, and the immunosuppressive microenvironment.

CAR Structure

CARs are modular synthetic receptors that consist of four main components: (1) an extracellular target antigen-binding domain, (2) a hinge region, (3) a transmembrane

domain, and (4) one or more intracellular signaling domains. Here we will discuss the current principles underlying CAR design.

Antigen Binding Domain

The antigen binding domain is the portion of the CAR that confers target antigen specificity. Historically, the antigen-binding domains are derived from the variable heavy (VH) and light (VL) chains of monoclonal antibodies, connected via a flexible linker to form a single-chain variable fragment (scFv). In order to recognize antigens on tumor cells, induce CAR signaling, and activate T cells, the CARs antigen binding affinity must be sufficiently high but not high enough to result in activation induced death of the CAR expressing T cell and trigger toxicities (discussed later in this review). While affinity is certainly one of the most important factors to further complicate matters, it has been shown that even scFvs with similar affinities can differentially impact CAR-T cell function.

Hinge Region

The hinge or spacer region is defined as the extracellular structural region that extends the binding units from the transmembrane domain. The hinge functions to provide flexibility to overcome steric hindrance and contributes to the length in order to allow the antigen-binding domain to access the targeted epitope. Importantly, the selected hinge appears to impact CAR functionality as differences in the length and composition of the hinge region can affect flexibility, CAR expression, signaling, epitope recognition, strength of activation outputs, and epitope recognition.

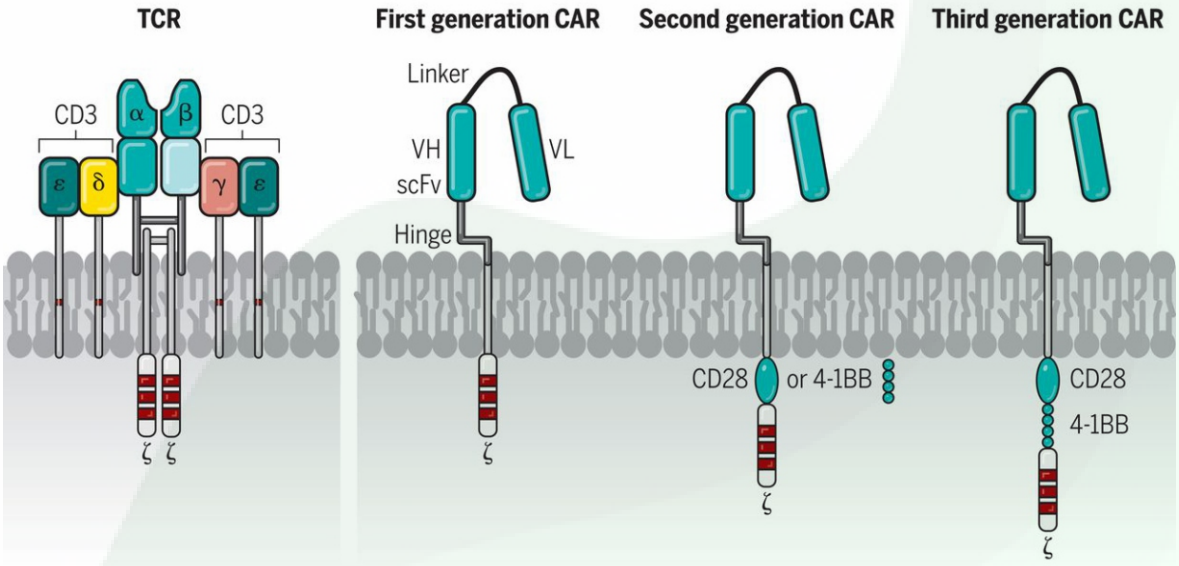
Transmembrane Domain

Among all of the components of CAR's, the transmembrane domain is probably the least characterized region. The major function of the transmembrane domain is to anchor the CAR to the T cell membrane, although evidence suggests that the transmembrane domain can also be relevant for CAR-T cell function. More specifically, studies suggest that the CAR transmembrane domains influence CAR expression level, stability, can be active in signaling or synapse formation, and dimerize with endogenous signaling molecules. Most transmembrane domains are derived from natural proteins including CD3ζ, CD4, CD8α, or CD28. The effect of one transmembrane compared to another on CAR function is not well studied as the transmembrane domain is frequently changed based on the requirements of the extracellular spacer region or the intracellular signaling domains.

Intracellular Signaling Domain(s)

Arguably the most attention in CAR engineering has been focused on understanding the effects of CAR co-stimulation with the goal of generating CAR constructs with the optimal endodomain. First generation CARs engineered in the late 1990s

contained a CD3ζ or FcRγ signaling domainA large majority of CARs rely on activation of CAR-T cells through CD3ζ derived immunoreceptor tyrosine-based activation motifsEffective T cell responses are not able to be generated by only signaling with these motifs .howeverThe durability and persistence of these first generation CARs are not robust in vitro.These findings were echoed by clinical studies that showed limited or no efficacy With this understanding of the importance of co-stimulation for durable CAR-T cell therapy, second generation CARs with one co-stimulatory domain in series with the CD3ζ intracellular signaling domain were generated. Clinically, second generation CAR-T cells have produced strong therapeutic responses in several hematological malignancies, including chronic lymphocytic leukemia, B-cell acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and multiple myeloma and the efficacy of second generation CAR-T cells are currently being investigated in solid tumors, including glioblastoma, advanced sarcoma, liver metastases, as well as mesothelioma, ovarian cancer, and pancreatic cancer. Preclinical studies of third generation CARs have produced mixed results. Specifically, CARs incorporating CD28 and 4-1BB signaling resulted in stronger cytokine production in lymphoma, and pulmonary metastasis showed an improved in vivo antitumor response compared to second generation CARs. In leukemia and pancreatic cancer models, third generation CARs showed no in vivo treatment benefits and failed to outperform second generation CARs in their respective models.



Article 2:

Chimeric antigen receptor T-cell therapy: An Overview of Generation of CAR T-cells, its advantages, challenges and opportunities in cancer treatment

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Key Words:

Lymphocytes, Chimeric antigen receptor, immunotherapy,

The immune system is the body's defense against infection and cancer. It is made up of billions of cells that are divided into several different types. Lymphocytes, a subtype of white blood cells, comprise a major portion of the immune system. There are three types of lymphocytes:

• B lymphocytes (B cells) make antibodies to fight infection.

• Tlymphocytes (T cells) have several functions, including helping B lymphocytes to make antibodies to fight infection, and directly killing infected cells in the body.

• Natural killer cells also attack infected cells and eliminate viruses.

Immunotherapy:

• Is a type of treatment that utilizes the body's own immune system to fight cancer?

• Improves the body's ability to detect and kill cancer cells.

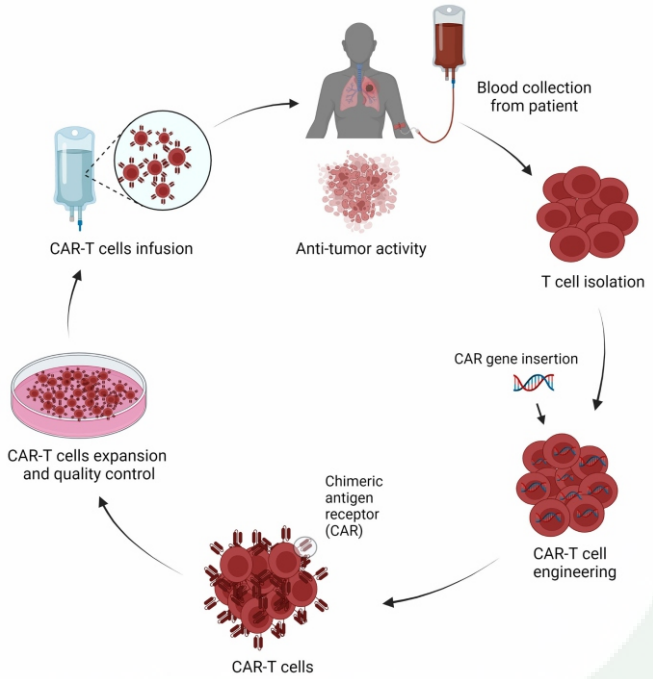
• Is based on the concept that immune cells or antibodies can recognize and kill cancer cells.

Immune cells or antibodies can be produced in the laboratory under tightly controlled conditions and then given to patients to treat cancer. Several types of immunotherapies are either approved for use or are under study in clinical trials to determine their effectiveness in treating various types of cancer.

Adaptive immunity, orchestrated by B-cells and T-cells, plays a crucial role in protecting the body from pathogenic invaders and can be used as tools to enhance the body's defense mechanisms against cancer by genetically engineering these immune cells. Several strategies have been identified for cancer treatment and evaluated for their efficacy against other diseases such as autoimmune and

infectious diseases. One of the most advanced technologies is chimeric antigen receptor (CAR) T-cell therapy, a pioneering therapy in the oncology field. With the advancement of CAR T-cell therapy, various attempts to cure lung cancer have continually emerged; yet choosing the optimal target remains a considerable challenge. An ideal target should be expressed with high coverage and specificity on lung cancer cells and not easily lost.

CAR T-cells generation: The concept behind CAR T-cells is deceivingly simple—uses a patient's own immune system to recognize and eliminate cancer cells. However, producing a CAR T-cell is a complex and multi-step process.The CAR, much like a native TCR, is a combination of protein domains that work in concert to identify the antigen of interest and then transmit a signal inside the T-cell that will ultimately lead to the destruction of the cancer cell.



The process for CAR T-cell therapy can take several weeks. First, white blood cells (which include T cells) are removed from the patient's blood using a procedure called leukapheresis. During this procedure, patients usually lie in bed or sit in a reclining chair. Two IV lines are needed because blood is removed through one line, the white blood cells are separated out, and then the blood is put back into the body through the other line. After the white cells are removed, the T cells are separated, sent to the lab, and altered by adding the gene for the specific chimeric antigen receptor (CAR). This makes them CAR T cells. These cells are then grown and multiplied in the lab. It can take several weeks to make the large number of CAR T cells needed for this therapy. Once enough CAR T cells have been made, they will be given back to the patient. A few days before the CAR T-cell infusion, the patient might be given chemotherapy to help lower the number of other immune cells. This gives the CAR T cells a better chance to get activated to fight the cancer. This chemotherapy is usually not very strong because CAR T cells work best when there are still some cancer cells to attack. Once the CAR T cells start binding with cancer cells, they start to increase in

start binding with cancer cells, they start to increase in number and can help destroy even more cancer cells.

Advantages of CAR T-Cell Therapy

Before CAR T-cell therapies came on the market, most patients with B-cell cancers depended upon chemotherapy and stem cell transplants. While the decision about an individual patient's treatment should remain with the patient and her/his health care provider, CAR T-cell therapy does offer certain advantages that could make it an appealing choice. Among these are the potential for shorter treatment times, prolonged durability, and fewer side effects.

Treatment Times

CART T-cell therapies require very short treatment times—generally a single infusion with less than 2 weeks of inpatient care. While chemotherapy treatment regimens typically also require months to complete, with multiple treatment cycles throughout (i.e., alternating periods of treatment and recovery, with generally 3 weeks set aside for recovery after each dose).

Durability

While the durability of CAR T-cell therapy is the subject of ongoing research, remission following CAR T-cell therapy appears to be long lived in many patients and commonly lasts for several years.

Safety

CAR T-cell therapies do not require aggressive chemotherapy, and unless there is an elevation in cytokines following infusion, patients receiving CAR T-cells do not typically require immunosuppressant. This is an important safety advantage over both stem cell transplantation and chemotherapy.

Challenges and side effects:

While CAR T-cell therapy can be a safer alternative to chemotherapy and even stem cell transplantation in certain patients, there are several common side effects of CAR T-cell therapy that can pose serious risks and therefore warrant careful monitoring. Cytokine release syndrome (CRS) is a common toxicity associated with CAR T-cell therapy. CRS results from overproduction of inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). These cytokines are produced when CAR T-cells proliferate in the body, indirectly activating macrophages and other immune cells. Neurologic toxicity is the second major side effect reported in patients after CAR-T cell infusion. Other possible and significant side effects of CAR T-cell therapy include (but are not limited to) infusion reactions, tumor lysis syndrome, cytopenias, cardiac toxicity, and hypo gamma globulinemia. While these and other side effects of CAR T-cell therapy can be serious or even life threatening, most will either resolve on their own or can be effectively managed with

drugs. In many cases, any side effects associated with CAR T-cell therapy will be less burdensome than those with chemotherapy.

Over the last decade, CAR-T cell therapy has revolutionized the treatment of hematological malignancies. The clinical application of CAR-T cell therapy and the identification of novel potential target antigens in cancer are subjects of ongoing research. Chimeric antigen receptor T cell clinical trials have generated impressive results in the early outcomes of CAR T-cell therapy patients with blood cancers.

Article 3:

An Introduction to Chimeric Antigen Receptor Its Limitation and Potential Strategies

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Interest in immune system activation as atherapeutic approach for treating cancer began in the late 19th century when William Coley injected heat-inactivated bacteria into the tumor mass , resulting in its mass reduction .

Chimeric antigen receptor (CAR) T-cell therapy represents a major advancement in modified cancer treatment. In this approach, a patient's own T cell are genetically engineered to express a synthetic receptor that binds a tumor antigen, CAR T cell are then expanded for clinical use and infused back into patient's body to attack and destroy chemotherapy resistant cancer.

LIMITATION OF CHIMERIC ANTIGEN RECEPTOR

There are several limitations of chimeric antigen receptor (CAR) therapy, which is a form of immunotherapy used to treat certain types of cancer. Here are a few notable limitations:

1. Limited applicability: CAR therapy has shown great promise in treating certain blood cancers, such as leukemia and lymphoma. However, its effectiveness in solid tumors is still being explored. CAR therapy may not be suitable or effective for all types of cancer.

2. Antigen escape: Cancer cells can evolve and develop mechanisms to evade CAR therapy. They may downregulate or lose the antigen targeted by the CAR, rendering the therapy less effective over time. Antigen escape can lead to relapse or resistance to treatment.

3. On-target, off-tumor toxicity: CARs are designed to target specific antigens expressed on cancer cells. However, some antigens can also be present on normal cells, which may lead to off-target effects. This can result in unintended damage to healthy tissues and organs, causing adverse side effects.

4. Manufacturing and scalability challenges: CAR therapy involves genetic modification of a patient's immune cells, which requires a complex and costly manufacturing process. Quality control, scalability, and logistical challenges can pose limitations to widespread adoption of CAR therapy.

5. Safety concerns: Although CAR therapy has shown promising clinical outcomes, there have been reports of severe side effects, including cytokine release syndrome and neurotoxicity. These safety concerns highlight the need for continuous monitoring and management of potential adverse events.

It's important to note that research and advancements in CAR therapy are ongoing, and these limitations are subject to change as new discoveries are made.

POTENTIAL STRATEGIES

Some potential strategies involving chimeric antigen receptor (CAR) technology:

1. Targeting specific antigens: CAR-T cell therapy can be designed to target specific antigens expressed on the surface of cancer cells. By developing CARs that recognize and bind to these cancer-specific antigens, the therapy can selectively target and eliminate cancer cells while sparing healthy cells.

2. Boosting CAR-T cell persistence: CAR-T cell therapy can be enhanced by engineering CAR-T cells to have increased longevity and persistence in the body. This can be achieved by incorporating co-stimulatory molecules into the CAR design or by modifying the CAR-T cell production process.

3. Overcoming immunosuppression in the tumor microenvironment: Tumors often create an immunosuppressive microenvironment, which hampers CAR-T cell activity.

4. Incorporating additional therapeutic elements: Researchers are investigating the possibility of engineering CAR-T cells to secrete additional therapeutic molecules, such as cytokines or antibodies, to further enhance their anti-tumor activity.

5. Reducing off-target toxicity: Efforts are being made to minimize the potential for off-target toxicity by refining the design and specificity of CARs. Researchers are developing strategies to improve the selectivity of CAR-T cells, ensuring that they only target cancer cells and minimize damage to healthy tissues.

These are just a few potential strategies that are being explored to optimize the effectiveness and safety of CAR-T cell therapy. Ongoing research and advancements in CAR technology hold promise for further improving this innovative cancer treatment approach.

CONCLUSION

In conclusion, while chimeric antigen receptor (CAR) therapy has shown significant potential in treating certain types of cancer, such as blood cancers, it has limitations that need to be considered. One notable limitation is its limited applicability, as its effectiveness in solid tumors is still being studied. It is important to continue exploring and developing alternative therapies to address the diverse needs of patients with different types of cancer.

Article 4:

Review Studies of Chimeric Antigen Receptor T- Cell an Advance Cell Therapy for Cancer

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Chimeric antigen receptors (CARs)—also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors—are receptor proteins that have been engineered to give T cells the new ability to target a specific antigen . The receptors are chimeric in that they combine both antigen-binding and T cell activating functions into a single receptor.

A first generation CAR containing a CD4 extracellular domain and a CD3ζ intracellular domain was used in the first clinical trial of chimeric antigen receptor T cells by the biotechnology company Cell Genesys in the mid 1990s

cells are genetically engineered to express chimeric antigen receptors specifically directed toward antigens on a patient's tumor cells, then infused into the patient where they attack and kill the cancer cells. Adoptive transfer of T cells expressing CARs is a promising anti-cancer therapeutic, because CAR-modified T cells can be engineered to target potentially any tumor associated antigen.

Immunotherapy is a type of biological therapy. Biological therapy is a type of treatment that uses substances made from living organisms to treat cancer.

Several types of immunotherapy are used to treat cancer

1. Immune checkpoint inhibitors
2. T-cell transfer therapy
3. Monoclonal antibodies
4. Treatment vaccines
5. Immune system modulators

T-cell transfer therapy (CAR-T cell therapy)

There are two main types of T-cell transfer therapy: tumor-infiltrating lymphocytes (or TIL) therapy and CAR T-cell therapy.

CAR binding to target antigen expressed on the cell surface to help them achieve the target CAR is made up of three other proteins, one protein that recognize antigens on the cancer cell. And other two proteins that signal the T-cell to activate when that first protein attaches to an antigen on the cancer cell. Activated CAR T- cell multiply and

signal to other parts of the immune system .these signaling proteins are known as cytokines.

Some FDA approved CAR T-cell therapies

Brexucabtagene Autoleucel targeted antigen Cd19

Tisagenlecleucel targeted antigen Cd19

Citacabtagene autoleucel targeted antigen BCMA

Axicabtagene Ciloleucel targeted antigen Cd19

CAR T- cell therapy works:

Collection: T cells are collected from patients via apheresis, a process that withdraws blood from the body, and moves through a cell separator to collect the needed blood components. Researchers have also begun to rethink the source of immune cells for CAR T-cell therapies—using T cells collected not from patients, but from healthy donors.

Engineering: The T cells are sent to a laboratory where they are genetically engineered to target a specific type of cancer.

Multiplication: The genetically modified T cells are "expanded" by growing cells in the laboratory until there are millions of them. The process of engineering and growing sufficient quantities of CAR T cells can take a few weeks. When there are enough of them, the CAR T cells are frozen and sent to the hospital or center where the patient is being treated..

Conditioning Therapy: Prior to infusion of the CAR T cells, patients may receive chemotherapy for their cancer. This helps to create space in your immune system for the infused CAR T cells to expand and proliferate.

Infusion: When the CAR T cells are ready, the cells are infused through a central line, in a process similar to a blood transfusion. Patients may receive medications to prevent and control possible side effects of the newly-engineered cells. Patients may receive their CAR T cells in the hospital or in the outpatient clinic.

CAR T- cell qualities

Fourth generation CARs (also known as TRUCKs or armored CARs) further add factors that enhance T cell expansion, persistence, and anti-tumoral activity. This can include cytokines, such is IL-2, IL-5, IL-12 and co-stimulatory ligands

Proliferative ability

Cytotoxic potential

Capacity to produce critical effector cytokines.

Capability of resisting immunosupression.

About Sagar Group and SIPTec

Sagar group came into existence in the year 1983 under the visionary leadership of Chairman Shri Sudhir Kumar Agrawal. Over the years, it has now transformed into one of the largest corporate house and business conglomerate of Central India. In its journey of over three decades, the group has successfully ventured in the field of education, real estate, production and manufacturing to employ 5000+ people and impact lives of more than two lakh people every day. **Sagar Group** has been felicitated with IBC24 Excellence Award 2017 for its contribution to Madhya Pradesh's Industrial Development and Incredible Societal Development. **Agrawal Builders** have established its presence as one of the leading Real Estate giants with over 40 years of rich experience in building state-of-art residential projects. **Sagar Manufacturers Pvt Ltd** has pledged to use the best fibers to produce superior quality yarns with the world-class production technology. In a short span of time the company has achieved an installed capacity of 2,00,000 spindles and exporting its products to over 20+ countries. **Sagar Nutriments Pvt Ltd is Sagar Group's** recent venture in food processing premium quality basmati rice.

Sagar Group has earned a lot of praise across the nation empowering youth of Madhya Pradesh with a bright career and life. The group provides world class school and technical education under **Sagar Group of Institutions** to 20000+ students with 2000+ dedicated faculties. The group imparts schooling through the chain of **Sagar Public Schools (SPS(** to nurture the young mind. Today, SPS is considered as the most preferred brand forholistic education and Indian Value System to its core featuring amongst the **Top 100 schools in India** with its campuses at **Saket Nagar, Gandhi Nagar, Rohit Nagar, Ratibad, Katara Extension and Dwarka Dham. Sagar Group of Institutions** are engaged in providing the best technical education in the field of engineering, pharmacy, and management.

Sagar Institute of Pharmacy and Technology (SIPTec) is the premier institution known for its high standards in teaching and research in pharmaceutical sciences. SIPTec was established in 2008. The Institute is also registered under **CCSEA**. Today, within a short span of 14 years, the institute has gained a reputation of being one of the **top Pharmacy Colleges in MP** that provides total pharmaceutical education comprising B.Pharm. and M.Pharm. (Pharmaceutics & Pharmaceutical Chemistry).

About Sagar Multispeciality Hospital (SMH)

Sagar Multispeciality Hospital (SMH), located on Hoshangabad Road, is a magnificent 300-bed facility situated in the heart of Bhopal. Our unwavering determination is to become the leading Multispeciality medical facility, catering to the healthcare needs of Central India and its surrounding areas. At Sagar Multispeciality Hospital, we offer the unique advantage of integrated medical care in a multidisciplinary setting, all conveniently located under one roof. Our esteemed faculty consists of highly qualified doctors, nurses, and healthcare professionals, ensuring that you receive the highest level of expertise and care available. Get ready to embark on a journey of exceptional healthcare at Sagar Multispeciality Hospital, where your well-being is our utmost priority.

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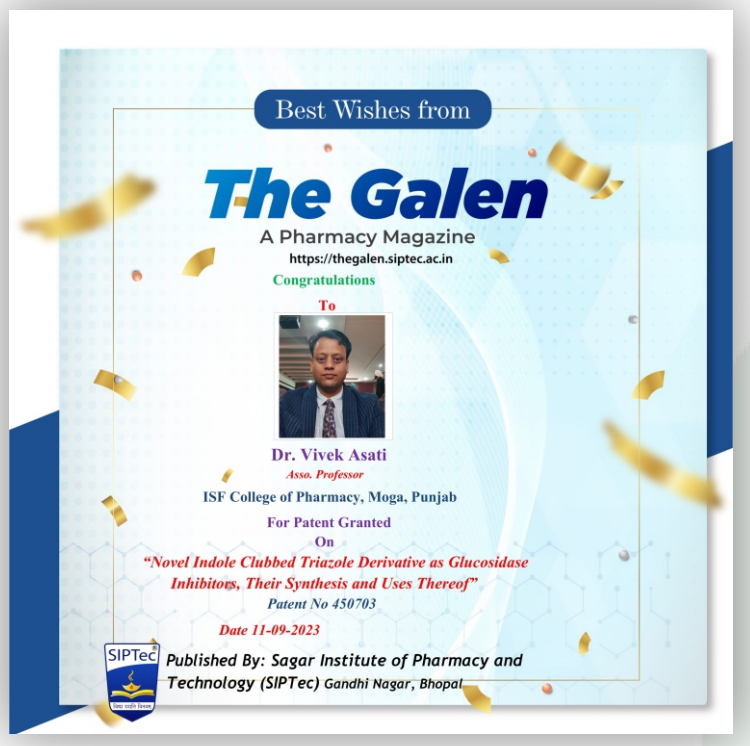

Best Article Winner of 4th Edition of this Magazine (Selected by Editorial Committee of The Galen)

Dr. Anupam Kumar Pathak
Retired Professor
Department of Pharmacy, Barkatullah University, Bhopal, M.P.

Title of Article: Chimeric Antigen receptor T-cell Therapy for Cancer

Next Theme:
Parasitic Diseases, Its Precautions and Treatment

Deadline for article submissions:
31st December 2023