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Chapter 2

In vitro and in vivo anticancer agents immunotherapy

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1 Introduction

Immuno-oncology has revolutionized the treatment of cancer patients. Sir William Coley, known as the father of immunotherapy, was the first to explore the potential of the immune system in treating cancer. His pioneering work was the first to show that tumors could be recognized and attacked by the immune system. Coley developed a bacterial mixture, combining *Streptococcus pyogenes* and *Serratia marcescens*, hoping to induce sepsis and trigger a strong immune and anti-tumor response. This marked the earliest documented attempt at an anti-cancer treatment. He believed that by stimulating the immune system, tumors could be fought and reduced. His approach followed a logical progression: he researched existing treatments, created a new therapy using bacterial toxins, tested it on his patients, and published his results in *Annals of Surgery* in 1891. However, the underlying mechanism of this treatment remained unclear. Due to this, the medical community shifted towards surgical and radiotherapy options in the early 20th century.

A new chapter in cancer immunotherapy emerged with the discovery of innate immune cells capable of eliminating cancer cells. The identification of immune checkpoint molecules such as CTLA-4 and PD-1 further advanced immuno-oncology, earning Dr. James Allison and Dr. Tasuku Honjo the Nobel Prize in Physiology or Medicine in 2018. More recently, checkpoint inhibitors have been developed and introduced into clinical practice, significantly altering both the medical and commercial landscapes of cancer treatment. Some examples of checkpoint inhibitors include Imbruvica, OPDIVO, and Keytruda. Of these, Keytruda has achieved the highest sales ranking, among the top 15 drugs worldwide. Checkpoints function like switches that must be activated or deactivated

to trigger an immune response. These checkpoint inhibitors, also referred to as monoclonal antibodies, are specifically engineered to target checkpoint proteins.

This chapter will highlight key milestones in the history of immunotherapy research, provide an overview of its current state, and offer a glimpse into the future of this evolving field.

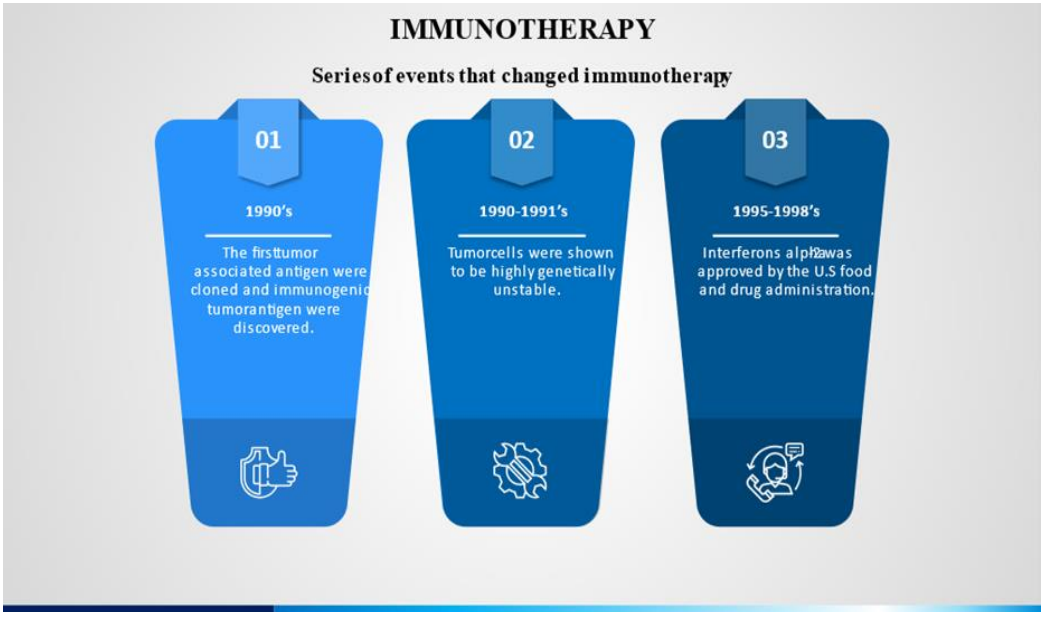

Fig.1: Series of events that changed Immunotherapy

2 Past of Immunotherapy

The principal agents in this group are cytokines, which include interleukins, interferons, and chemokines. During the 1990s, several pivotal developments transformed the field of immunotherapy. These developments included:

The cloning of the first tumor-associated antigen and the identification of immunogenic tumor antigens, indicating that the immune system could potentially recognize and eliminate them (Petroni et al., 2021; Santoni et al., 2023; Atallah-Yunes & Robertson, 2022).

It was demonstrated that tumor cells exhibit a high degree of genetic instability.

In 1995, Interferon-α2 (IFN-α2) received approval from the U.S. Food and Drug Administration (FDA) as an adjuvant therapy for stage IIB/III melanoma. Following this,

in 1998, the FDA approved IL-2 for the treatment of metastatic melanoma and renal cell carcinoma.

2 Present of Immunotherapy

Cytokines, such as interleukin-2, were first identified in 1976 as the supernatants from activated human T cells. These cytokines play a crucial role in triggering active immune responses against tumors, as well as in the negative regulation of immune activity to ensure homeostasis and self-tolerance. Self-tolerance is primarily controlled by two key types of interleukins: CD4+FoxP3+ Tregs. A critical area of ongoing research is understanding how cytokines regulate the generation and maintenance of Tregs, and how to disrupt this aspect of tolerance to foster effective and sustained anti-tumor immunity.

They are secreted by nearly every cell in the body and are predominantly involved in cellular immune responses against viral infections. 14 The only member of this family is IFN-γ, which binds to a distinct receptor complex (IFN R1 and IFN R2).

The Type II IFN receptor is a subset of the type II cytokine receptors.This recently-discovered family consists of IFN- gamma 1, IFN- gamma 2, and IFN- gamma 3 which activate an IL-10 receptor 2 subunit and IL-28 receptor subunit complex.

Vaccines like Canvaxin initially showed promise in phase III clinical trials but ultimately did not provide any benefit to patients. The therapeutic polyvalent cancer vaccine, Canvaxin™ (CancerVax Corp., Carlsbad, CA), is a polyvalent formulation designed for cancer treatment. Another approach to vaccine therapy is through dendritic cell vaccines. Dendritic cells are essential immune cells that process antigens and present them to naive T cells, initiating the adaptive immune response against tumors. The first clinical trial involving ex vivo dendritic cells took place in 1996. T cells play a critical role in the adaptive immune response to cancer, and after dendritic cells process tumor antigens, they interact with T cells, leading to the activation of these T cells, which then work to eliminate the tumor. The first T-cell-based therapy was reported by Rosenberg in 1988. A particularly noteworthy approach is chimeric antigen receptor (CAR) therapy, which allows T cells to recognize tumor antigens through an antibody, and the T cell becomes activated via the intracellular TCR signaling domain.

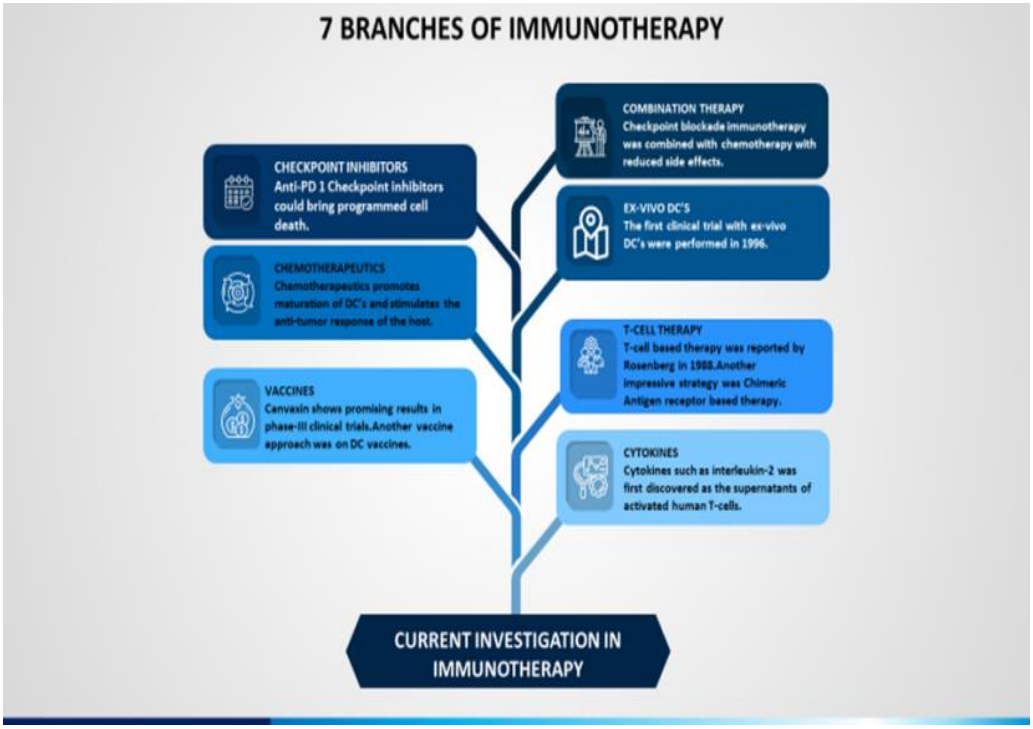

Fig.2 : The seven branches of Immunotherapy

3 Future of Immunotherapy

Transformer-based models such as BERT have caused a significant change in the field of NLP and consistently establish higher levels of performance. These models use self-attention mechanisms to better capture contextual information compared to traditional RNNs and CNNs (Cai et al., 2021; Sun et al., 2023; Rizzo et al., 2021).

In patients undergoing immunotherapy with checkpoint inhibitors, predictive markers such as absolute lymphocyte count have been linked to the effectiveness of these treatments. Research into predictive markers for the efficacy of immune checkpoint inhibitors has increasingly focused on understanding intermolecular interactions. Studies have shown that immunotherapy shares similarities with molecular targeted therapy, as both approaches offer specific benefits for patients with tumors (Zhu et al., 2021; Rizzo & Ricci, 2022; Lemaire et al., 2021; Zhu et al., 2022).

The use of combination therapy has become a standard approach in cancer treatment. For example, a platinum-based doublet for treating non-small cell lung cancer (NSCLC) has proven successful by combining cytotoxic chemotherapy agents with other drugs. Research has demonstrated that combining different immunotherapies is more effective than using a single treatment strategy. While the immune response is crucial in fighting cancer, mechanisms of immunosuppression can limit effective anti-tumor immunity. One such mechanism is the dysfunction or exhaustion of immune cells in tumor-bearing individuals. Another study highlighted that combining PD-1/PD-L1 blockade with CTLA-4 inhibition allows tumor-specific T cells, which would typically be inactivated, to persist and expand, enabling them to perform their effector functions. This combination shifts the tumor microenvironment from a suppressive to an inflammatory state.

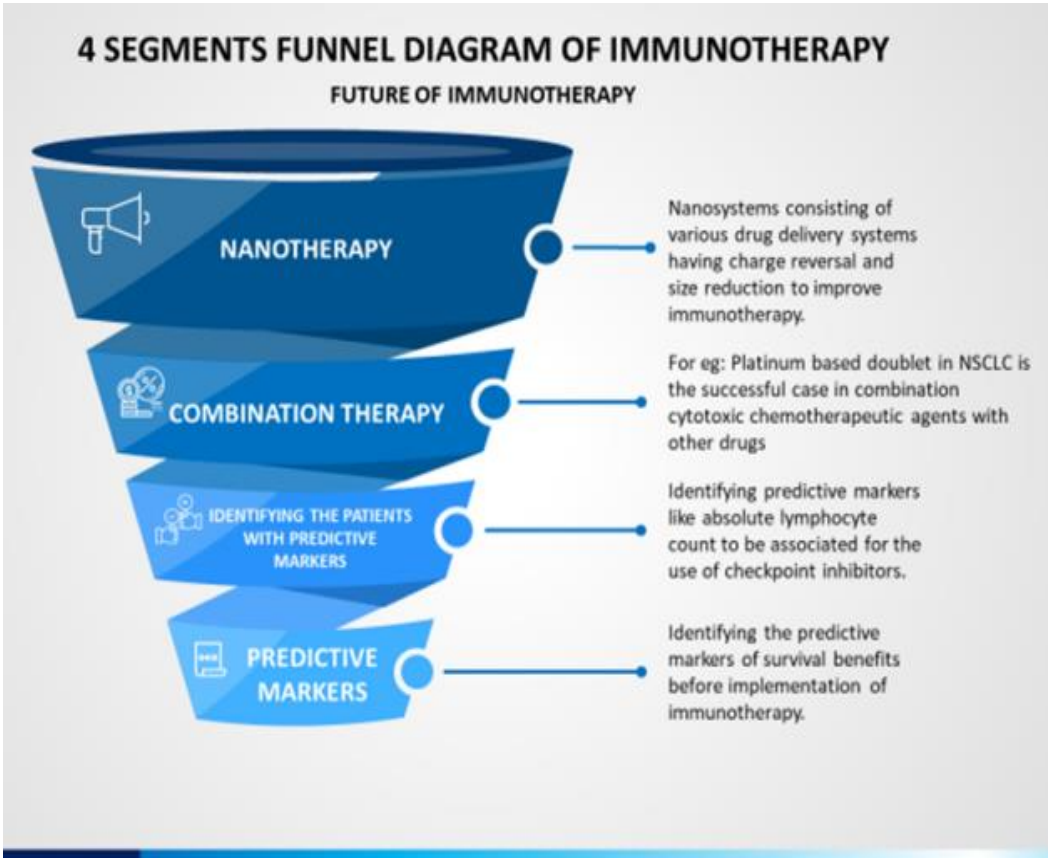

Fig. 3 : The future aspect of Immunotherapy

Nanotherapy has also been gaining attention in recent years. A recent study developed a programmable nanosystem that is based on dual pH/redox-responsive, size-shrinkable, and charge-reversal micelles. This system co-delivers NLG919 and CUR to enhance

chemotherapy and improve IDO immunotherapy. The system's ability to shrink in size and reverse its charge helps to overcome barriers to drug delivery, allowing for the efficient in vivo delivery of CUR and NLG919 in response to the weakly acidic tumor microenvironment.

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3 The Immune Checkpoint Inhibitors

The process is carefully regulated by immune checkpoints, which are surface receptors on immune cells that either activate or inhibit immune responses. Various types of checkpoint inhibitors, each targeting different checkpoints or "brakes" on immune cells, are currently being utilized. One of the most extensively researched classes of these inhibitors is the anti-PD-1/anti-PD-L1 category.

Immuno-oncology checkpoint inhibitors have revolutionized the oncology clinical landscape and market dynamics. Within the top 15 drugs by global sales, **Keytruda** leads as the most prominent therapy in oncology in terms of US dollar revenue. In the United States, clinical-stage assets reveal that immuno-oncology agents now account for a significantly larger share of the cancer treatment pipeline compared to non-immuno-oncology therapies. These agents also demonstrate remarkable success across all stages of clinical development—Phase 1, Phase 2, and Phase 3 trials—outperforming their non-immuno-oncology counterparts in terms of number and progression.

Not all immune checkpoints have the same therapeutic potential. For instance, the agonistic OX40 antibody demonstrates limited clinical efficacy, whereas the CD28 antibody, even at doses far below therapeutic levels, triggered severe cytokine release syndrome, necessitating intensive care for the first six healthy volunteers treated. This underscores the ongoing challenge in clinical research to identify the optimal combination of immune checkpoint inhibitors that achieves the desired level of immune activation without excessive risks. A recent meta-analysis revealed a fatality rate as high as 1 in 77 patients treated with combination immune checkpoint inhibitors.

PD-1inhibitors:

– Nivolumab (Opdivo®), made by Bristol-Myers Squibb (BMS), approved in 2015

– Pembrolizumab (Keytruda®), made by Merck, was approved in 2015.

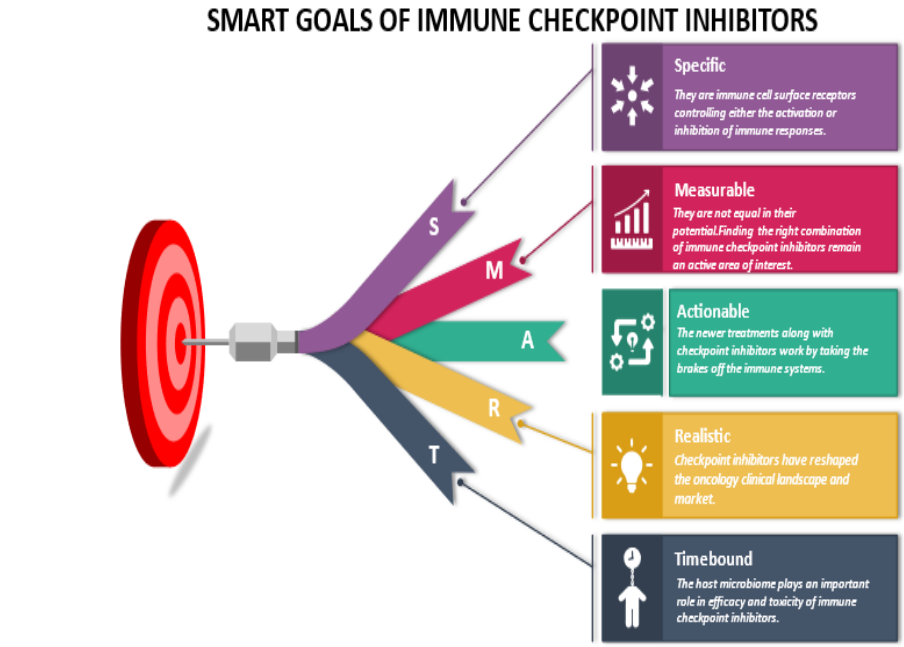

Fig.4 : Salient features of Immune Checkpoint Inhibitors

4 Newer agents of immunotherapy used in-vitro

The next generation immuno-oncology agents are not able to improve the widespread use of checkpoint inhibitors, Nanobiotix team had offered a new potential first in class radio enhancer which is also a powerful orthogonal approach to modulate the tumour microenvironment and augment checkpoint inhibitor and other immuno-oncology agents. The first in class nanoparticle having 50 nm in size is composed of crystalline hafnium oxide nanoparticle functionalised by a negative charged phosphate coating. These physicochemical properties are fundamental to its intratumor bioavailability and persistence in cancer cells.NBTXR3 radiated with radiotherapy in combination with anti-PD-1 checkpoint inhibitors had shown to improve the efficacy of the immune checkpoint inhibitors.

In two additional studies, nanoparticles loaded with dual drugs demonstrated significantly enhanced in vitro cytotoxicity against resistant cancer cell lines, offering a promising approach to overcoming the multidrug resistance (MDR) problem that has hindered numerous cancer therapies. Table 1 shows in vitro and in vivo anticancer agents' immunotherapy.

Table 1 In Vitro and In Vivo Anticancer Agents Immunotherapy

Sr No.	Agent Name	Type (In Vitro/In Vivo)	Target Cancer	Mechanism of Action	Clinical Trial Phase	Key Outcomes
1	Example Agent 1	In Vitro	Leukemia	Activation of T-cells	Phase I	Reduced tumor size; limited toxicity
2	Example Agent 2	In Vivo	Breast Cancer	Blockade of PD-1/PD-L1 pathway	Phase II	Improved survival rates
3	Example Agent 3	In Vitro	Melanoma	Enhancement of NK cell activity	Pre-clinical	Effective in reducing melanoma cells in lab settings
4	Example Agent 4	In Vivo	Lung Cancer	CAR-T cell therapy targeting specific tumor antigens	Phase III	High remission rates in treated patients
5	Example Agent 5	In Vitro	Colorectal Cancer	Immune checkpoint inhibitors	Phase II	Promising results in early trials
6	Example Agent 6	In Vivo	Pancreatic Cancer	Cytokine therapy to enhance immune response	Phase I	Shown to stimulate immune system against cancer cells
7	Example Agent 7	In Vitro	Ovarian Cancer	Targeting cancer stem cells	Phase I/II	Early evidence of effectiveness in targeting cancer roots
8	Example Agent 8	In Vivo	Prostate Cancer	Vaccine-based immunotherapy	Phase II	Positive immune response with manageable side effects

9	Example Agent 9	In Vitro	Skin Cancer	Modulating T-regulatory cells	Pre-clinical	Promising lab results in controlling tumor growth
10	Example Agent 10	In Vivo	Hodgkin Lymphoma	Antibody-drug conjugate targeting CD30	Phase III	Significant improvement in survival rates
11	Example Agent 11	In Vitro	Cervical Cancer	Inhibition of immune escape mechanisms	Phase I	Encouraging results in targeting hard-to-reach tumors
12	Example Agent 12	In Vivo	Multiple Myeloma	Bi-specific T-cell engagers	Phase II	Significant tumor reduction noted
13	Example Agent 13	In Vitro	Sarcoma	Gene editing to enhance immune recognition	Pre-clinical	Successfully modified immune cells to recognize sarcoma
14	Example Agent 14	In Vivo	Bladder Cancer	Immune system modulators	Phase I/II	Improved treatment tolerance and efficacy
15	Example Agent 15	In Vitro	Thyroid Cancer	Targeting specific oncogenic mutations	Pre-clinical	Shown potential in specific mutation profiles

5 In vivo agents used in immunotherapy

The inaugural clinical trial utilizing ex vivo dendritic cells (DCs) was conducted in 1996. As our knowledge of DC biology has advanced, a variety of innovative DC-based vaccine strategies have been developed. Palucka has indicated that DCs can be harnessed for cancer vaccines through multiple approaches, such as: (I) vaccines using nontargeted peptides/proteins and nucleic acids that DCs capture in vivo; (II) vaccines that consist of antigens directly linked to antibodies targeting DCs; or (III) vaccines using ex vivo prepared DCs that are charged with antigens. These DCs can be created ex vivo, equipped with various antigen types, activated, and administered to individuals in need. Over the past 15 years, clinical investigations have evaluated different DC vaccine formulations, various DC activators, diverse antigen forms ranging from simple peptides to complex whole-tumor-cell hybrids, and various DC administration routes. Initially, these studies

were executed as standalone therapies, but now they are being combined with other agents like systemic adjuvants to assess their efficacy.

Research has shown that the enzyme-activatable drug exhibits inadequate penetration when introduced in vivo. The effective delivery and therapeutic efficacy of PPM-DDS are severely restricted by poor tumor penetration and cellular uptake due to the various biological barriers present in vivo. These findings confirm the viability of discovering novel immunotherapy targets in intricate tissue environments in vivo. Our results demonstrate the potential to identify genes that behave differently across tissues, as seen with T-cell accumulation in tumors versus secondary lymphoid organs.

The advent of nanotechnology has undeniably helped overcome many challenges in the quest to develop the "magic bullet," yet significant efforts are still necessary. Future enhancements will likely focus on achieving specific site targeting, maintaining the agent for the desired duration, and enabling controlled release timing. Additionally, it is anticipated that more advanced and multifunctional systems will be developed, incorporating new materials like stimuli-responsive polymers. These systems aim to tackle the dual challenges of in vivo diagnostics and on-demand drug release. Furthermore, the pursuit of strategies that emulate natural entities and leverage biomimetic principles is expected to drive substantial advancements in the coming years.

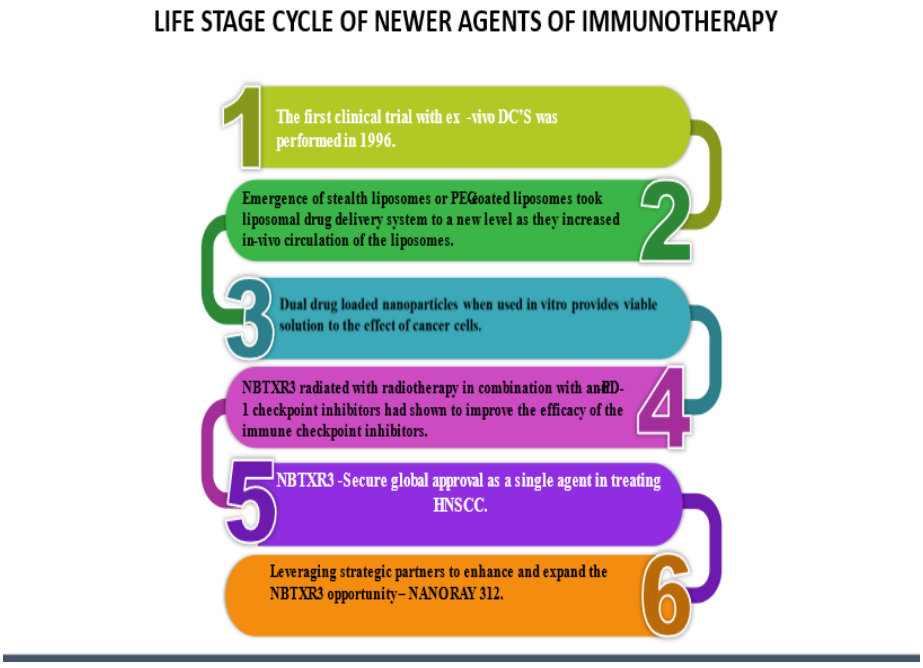

Fig.5 Life Stage cycle of Immunotherapy

Conclusion

Cancer immunotherapy has significantly improved both survival rates and quality of life for patients. However, cancer types vary widely, and reliable predictors of treatment response and toxicity remain scarce. Various strategies targeting different immune response mechanisms against tumors have been developed, each demonstrating some degree of antitumor efficacy. Among these, immunological checkpoint inhibitors stand out as a vital class of immunotherapeutic agents. Over time, the conventional assessment tools used during the era of chemotherapy and targeted therapies have proven to be less effective for evaluating these newer treatments.

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