PROJECT SEMESTER REPORT

On

T-Cell Immunotherapies Market (4th Edition), 2019-2030

(Project Semester February-May 2019)

"ROOTS ANALYIS Pvt. Ltd."

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I wish to express my sincere gratitude to **Mr. Gaurav Chaudhary**, CEO, for providing me an opportunity to do my internship and project work at "**Roots Analysis Pvt. Ltd.**".

I sincerely thank **Ms. Pemba Lahmo** for her constant inspiration, encouragement and guidance throughout the project. I also wish to express my gratitude to all the members of Roots Analysis who rendered their help during the period of my project work.

I would also like to express my sincere gratitude to my parents who are always constant source of inspiration to me.

SANJEEVANI RAVI SRIVASTAVA

CERTIFICATE

This is to certify that the work reported in the B.Tech. academic report entitled **"*T-Cell Immunotherapies Market (4th Edition), 2019-2030*"**submitted by **Sanjeevani Ravi Srivastava** in partial fulfillment for the award of degree of B.Tech. in Bioinformatics from **Jaypee University of Information & Technology, Waknaghat** has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of any other degree, diploma or such other titles.

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Senior Business Analyst
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DECLARATION

I hereby declare that the project work entitled ***"T-Cell Immunotherapies Market (4th Edition), 2019-2030"*** is an authentic record of my own work carried out at Roots Analysis Pvt. Ltd. as a requirement of six months project for the award of degree of Bachelor of Technology in Bioinformatics at Jaypee University of Information and Technology,Solan(H.P). under the guidance of **Ms. Pemba Lahmo** during the period, February 2019 to May 2019.

Sanjeevani Ravi Srivastava

Roll No – 151505

Date: May 23,2019

Certified that the above statement made by the student is correct to the best of our knowledge and belief. Roots Analysis owns the copyright of the findings presented in this report. Under no circumstances should this information be shared with other third parties without the prior consent of the company.

Pemba Lahmo

Senior Business Analyst

Roots Analysis Pvt. Ltd.

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1. SUMMARY

I was assigned a market report project on *"**T-Cell Immunotherapies Market (4th Edition), 2019-2030***". The report features an extensive study of the current market landscape and the future potential of T-cell immunotherapies (focusing particularly on CAR-T therapies, TCR therapies and TIL therapies). One of the key objectives of the study was to review and quantify the future opportunities associated with the ongoing development programs of both small and big pharmaceutical firms. Amongst other elements, the report features basic introduction of T-cell immunotherapy,prevalent and emerging trends related to T-cell immunotherapies as observed on the social media platform, Twitter.In addition, the report includes a detailed assessment of the current market landscape of T-cell immunotherapies with respect to type of therapies, type of developer (industry / non-industry), phase of development, target therapeutic indications, key target antigens, source of T-cells (autologous and allogenic), and route of administration. The reportalso includesanalysis of the partnerships that have been established in the recent past. Further the report includes discussion on price related to different cell-based therapies and the key promotional strategies that are being implemented by the developers of the marketed products.A detailed discussion on innovative technology platforms that are being used for the development of T-cell therapies, along with profiles of key technology providers.An extensive primary and secondary research were carried out to gather relevant information regarding the topic.

As a final outcome of the study, an excel database of close to 650T-cell therapies covering intensive details on several parameters was prepared. The parameters considered included type of therapies, type of developer (industry / non-industry), phase of development, target therapeutic indications, key target antigens, source of T-cells (autologous and allogenic), route of administration, dosage, current patient segment, therapeutic areas.

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2. COMPANY PROFILE

Roots Analysis Pvt. Ltd. is a business research and consulting firm which specializes in providing in-depth business research and consulting services for pharmaceutical industry. Focused on providing an informed and impartial view on key challenges facing the industry, the research is primarily driven by an in-depth analysis covering the following parameters:

- Research and development
- Existing market landscape
- Future Commercial potential
- Regulatory concerns
- Regional growth drivers
- Risks and opportunities

The firm specializes in analyzing areas which have lacked quality research so far or require more focused understanding within the broader industry. Apart from writing reports on identified areas, the company also provide bespoke research / consulting services dedicated to serve our clients in the best possible way.

The business reports highlight trends ranging from commercial success / potential, technological developments and outlook built around opportunities and threats. The company majorly focus on areas spanning the following domains:

- Therapeutic segments
- Emerging technologies
- Medical devices
- Drug Delivery
- Clinical Trials

2.1. RESEARCH METHODOLOGY

Most of the data presented in this report has been gathered via secondary and primary research. We have conducted interviews with experts in the area (academia, industry, medical practice and other associations) to solicit their opinions on emerging trends in the market. This is primarily useful for us to draw out our own opinion on how the market will shape up across different regions and technology segments. Where possible, the available data has been checked for accuracy from multiple sources of information.

The secondary sources of information include:

- Company's Annual reports
- Investor presentations
- SEC filings
- Industry databases
- News releases from company websites
- Government policy documents
- Industry analysts' views
- Research articles; Blogs; Press articles
- Company website

While the focus has been on providing a comprehensive view on the ongoing research, the report ***"T-Cell Immunotherapies Market (4th Edition), 2019-2030"*** also provides an independent view on research and development and future commercial potential emerging in the industry. This opinion is solely based on our knowledge, research and understanding of the relevant market gathered from various secondary and primary sources of information.

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3. WORK PROGRAM

The course of my internship at Roots Analysis started on 4th February 2019. I was assigned an individual project, on which I have worked for four months. The training program was structured as follows:

- The main objective of this report is to build a comprehensive pipeline of T-cell therapies by using available data from https://clinicaltrials.gov, company's website, LinkedIn profiles and other publicly available sources.
- Introduction chapter on T-cell Immunotherapy
- An analysis depicting prevalent and emerging trends related to T-cell immunotherapies as observed on the social media platform, Twitter.
- Collection of various partnerships and collaborations related to T-cell Immunotherapies.
- An elaborate discussion on various factors that form the basis for the pricing of cell-based therapies.
- A review of the key promotional strategies that have been adopted by the developers of the marketed T-cell therapies, namely Kymriah and Yescarta.
- A detailed discussion on innovative technology platforms that are being used for the development of T-cell therapies, along with profiles of key technology providers

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4. INTRODUCTION TO T-CELL IMMUNOTHERAPIES

4.1. CHAPTER OVERVIEW

Cancer is known to be one of the leading causes of death worldwide, accounting for 0.6 million deaths in 2017 in the US alone. The World Health Organization (WHO) states that the number of new cancer cases globally is expected to rise by 70% in the coming 20 years.[1, 2, 3] Although cancer therapeutics continue to be one of the most active areas in terms of drug development, there is still a significant unmet need in this domain. Conventional cancer treatments, such as chemotherapy, surgery and radiation therapy, have demonstrated very limited efficacy in late-stage cancers. Specifically, chemotherapy and radiation therapy are also associated with several side effects. Their non-specific nature has severe detrimental effects on the patients' quality of life.[4]

Amidst the current initiatives to develop more targeted anti-cancer therapies, immunotherapy has emerged as a highly potent option to eradicate tumor cells with minimal side effects. Immunotherapies essentially make use of the body's own immune system or its components to fight cancer. It is a relatively new concept, with the only success being targeted antibody based therapeutics, including monoclonal and conjugated antibodies. However, over the years, a number of different classes of immuno-therapeutics have emerged for the management and treatment of cancer; these include therapeutic cancer vaccines, oncolytic viruses, cytokines, immune checkpoint molecules and other whole cell-based therapies (adoptive cell therapies).[5]

This chapter talks about the general concepts related to immuno-therapeutics and offers additional insights on their potential in being used for the treatment of various oncological indications.

[1]Source:http://www.who.int/mediacentre/factsheets/fs297/en/
[2]Source: https://www.cancer.org/research/cancer-facts-statistics/global.html
[3]Source: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2017.html
[4]Source:https://www.nature.com/articles/icb20179.pdf?origin=ppub
[5]Source: http://jcmtjournal.com/article/view/2275/1732

4.2. PILLARS OF CANCER THERAPY

Cancer treatment has gone through a gradual development process. As indicated earlier, surgery is one of the conventional forms of cancer treatment and is still considered to be a vital part of the current standard of care. It is an efficient method to eliminate benign tumors that have not spread to different sites in the body. Surgery is primarily used to remove the entire tumor; however, it very rarely results in a complete cure since tracing all the sites to which the tumor may have metastasized is difficult.[6]

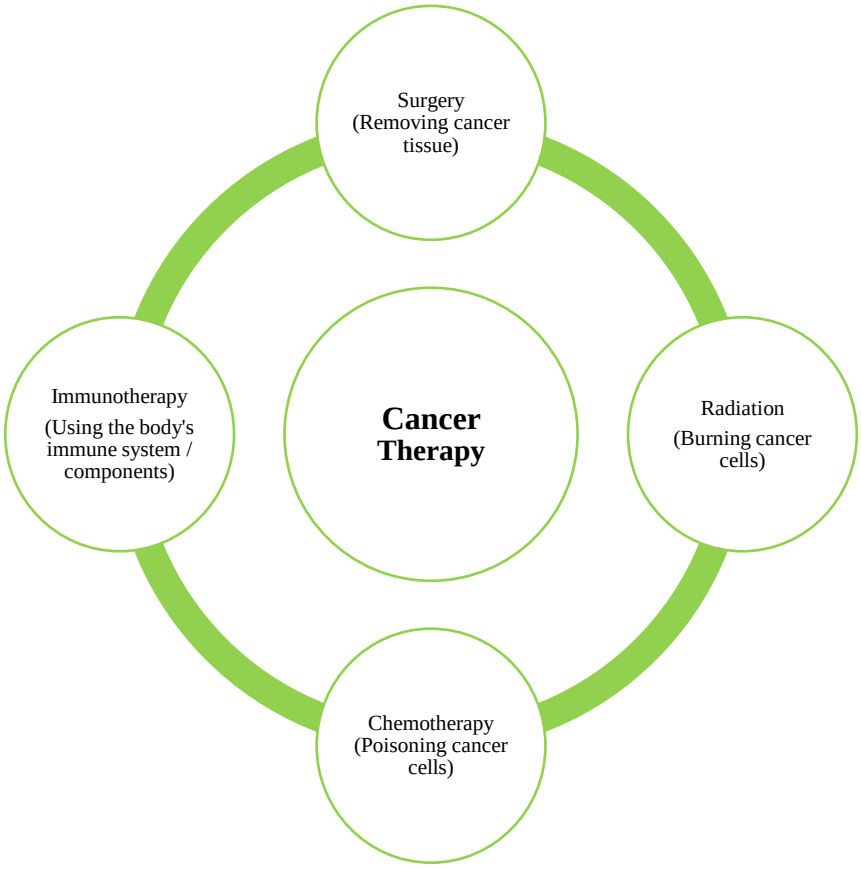
The advent of radiation therapy was witnessed in 1896 when a German professor, named Wilhelm Conrad Roentgen,[7] delivered a lecture, titled *Concerning a New Kind of Ray* (X-ray). A few months later, methods were devised to use X-rays for the elimination of cancer and soon radiation therapy came into being. Over the years, several developments in radiation physics and computer technology took place, making it possible to deliver radiations more precisely onto tumor sites. However, this type of treatment was shown to cause a number of side effects as well; the cytotoxic radiations used also affected all rapidly dividing normal cells in the target area, leading to a various complications post treatment.[8]

During World War I, the Germans used mustard gas as an agent of chemical warfare. Later, the compound was shown to possess potent hematopoiesis suppressor properties. A similar compound, called nitrogen mustard, was found to be effective in treating lymphoma. Soon after, Sidney Farber[9] demonstrated that the use of aminopterin led to disease remission in children with acute leukemia. With time, the use of cytotoxic chemicals, which came to be known as chemotherapy, emerged as a potent cancer treatment option. However, this form of therapy was also associated with its own set of side effects, owing to the harmful effect of the potent chemicals used for this purpose.[10]

Over the past decade, a number of advances in immunology led to a better understanding of the role of the immune system in cancer prevention. As a result, a number of therapies, aimed to harness the innate potential of the immune system to selectively eliminate cancer cells, have emerged. Cancer immunotherapy is currently classified among the pillars of modern cancer therapies. Figure 4.1 provides an illustrative summary of the four pillars of cancer therapy.

[6]Source: https://www.cancer.gov/about-cancer/treatment/types/surgery
[7]Wilhelm C Roentgen produced and detected the electromagnetic form of rays called Roentgen Rays (now called the X-Rays) and was awarded the Nobel Prize in Physics for the same
[8]Source: https://www.cancer.org/cancer/cancer-basics/history-of-cancer/cancer-treatment-radiation.html
[9]Sidney Farber, an American pediatric pathologist, is regarded as the Father of Modern Chemotherapy
[10]Source: http://www.cancer.org/cancer/cancerbasics/thehistoryofcancer/the-history-of-cancer-cancer-treatment-chemo

Figure 4.1 **The Four Pillars of Cancer Therapy**



Source: Roots Analysis, https://medium.com/@FUSFoundation/focused-ultrasound-can-help-propel-the-cancer-moonshot-456c689780c2

4.3. IMMUNOTHERAPY, AN EMERGING THERAPEUTIC OPTION

Conventional cancer treatments, such as chemotherapy, surgery and radiation therapy, have demonstrated very limited efficacy in late-stage cancers. Additionally, as mentioned above, chemotherapy and radiation therapy are associated with several side effects. Such treatment options usually destroy large populations of healthy and rapidly proliferating cells, along with the tumor cells. Their non-specific nature has severe detrimental effects on the patients' quality of life. Therefore, there is an urgent unmet need of innovative and effective cancer treatments for patients with late-stage and refractory cancer. Amidst the widespread initiatives to develop more targeted anti-cancer therapies, immunotherapy emerged as a highly specific and potent option to eradicate tumor cells with minimal side effects.

As indicated earlier, harnessing the underlying potential of the immune system to fight progressive diseases, such as cancer, forms the principle behind immunotherapy. Such therapies aim to educate the immune system with the knowledge of tumor antigens, thereby,

stimulating its effector to attack those cells that contain the specific target antigens.[11] Immunotherapies are known to provide therapeutic benefits by one of the following mechanisms:[12]

- Increasing adaptive immunity
- Decreasing immune suppression
- Increasing T-cell modulation activities

Moreover, immunotherapy may prevent recurrence of tumour post-surgery. The FDA-approved immunotherapies, such as Provenge[13]and Yervoy[14], represent milestones in the field of cancer immunotherapy for advanced prostate cancer and metastatic melanoma, respectively. Cancer immunotherapy has become an important treatment modality in treating cancer patients with advanced or refractory disease. It is important to mention that owing to the fact that this form of therapy involves the highly specific targeting capabilities of the immune system, it also has potential applications in a vast array of disease indications (other than oncology), including asthma, allergy and Alzheimer's disease. Cancer immunotherapy was called *Breakthrough of the Year* in 2013 due to the promising results obtained from the use of genetically modified T-cells to target cancer. The cancer immunotherapy market is currently a segmented market, comprising of both large and small pharma players. The relatively high efficacy of currently available immunotherapies has prompted several investors to fund initiatives in this field. Additionally, the existence of numerous unexplored avenues of research in this domain have caused new players, comprising of a mix of both novice and established stakeholders in the industry, to enter into this market.[15]

4.4. FUNDAMENTALS OF CANCER IMMUNOTHERAPY

Once the body recognizes an entity as foreign, it is capable of mounting an immune response against it that ultimately results in the selective elimination of the foreign entity. Moreover, the immune system retains a memory of the event and has the capability to keep it from relapsing. Immuno-oncology involves the study of the above phenomenon in order to develop novel treatment options that leverage innate potential of the immune system to treat disease in a specific manner and prevent it from recurring. Immunotherapies have been shown to stimulate the immune system in the following ways:[16]

- Blocking immune inhibitory signals

[11]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4372895/
[12]Source: http://www.sciencedirect.com/science/article/pii/S0093775414001973
[13] Provenge® is a registered trademark of Dendreon
[14] YERVOY® is a registered trademark of Bristol-Myers Squibb
[15]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4372895/
[16]Source: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-016-0623-5

- Activating immunostimulatory pathways
- Stimulating components of the innate and adaptive immune systems to elicit a disease-specific immune response

Table 4.1 provides a summary of the different types of immunotherapies available in the market.

Table 4.1 **Different Types of Immunotherapies and Their Mechanism of Action**

S. No.	Immunotherapy Type	Basic Mechanism	Advantages	Disadvantages
1	Cytokines (IL-2, IFN-α)	▪ Stimulation of the host's immune system	▪ Durable responses	▪ Low response rates ▪ Significant risk of serious systemic inflammation ▪ High-dose toxicity
2	Cancer vaccines	▪ Stimulation of the host's immune system	▪ Minimal toxicity ▪ Administered in the outpatient clinic	▪ Lack of universal antigens and ideal immunization protocols lead to poor efficacy and response
3	Adoptive cellular therapy	▪ Omits the task of breaking tolerance to tumor antigens	▪ Produces a high avidity in effector T-cells ▪ Lymphodepleting conditioning regimen prior to TIL infusion enhances efficacy ▪ Genetic T-cell engineering broadens TIL to malignancies other than melanoma	▪ Restricted to melanoma ▪ Safety issues, serious adverse effects, and lack of long lasting responses in many patients ▪ Requires time to develop the desired cell populations ▪ Expensive
4	Immune checkpoint inhibitors	▪ Releases pre-existing anticancer T-cell responses and possibly triggers new	▪ Exhibits potent antitumor properties ▪ Prolongation of overall survival ▪ Sufficient clinical responses, which are often long-lasting ▪ Therapeutic responses in patients within a broad range of human cancers	▪ Only a relatively small fraction of patients obtains clinical benefit ▪ Severe immune-related adverse events have been observed in up to 35% of the patients
5	Combination immunotherapy (immune checkpoint inhibitors as the backbone)	–	▪ Improvement of anti-tumor responses / immunity	▪ May lead to increase in the magnitude, frequency, and onset of side effects

Source: Roots Analysis, https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-016-0623-5

4.5. Classification of Cancer Immunotherapies

4.5.1. By Mechanism of Action

Based on the mechanism of action, there are two main classes of cancer immunotherapies, namely active and passive immunotherapies. Further details on these classes are highlighted in the following sections.

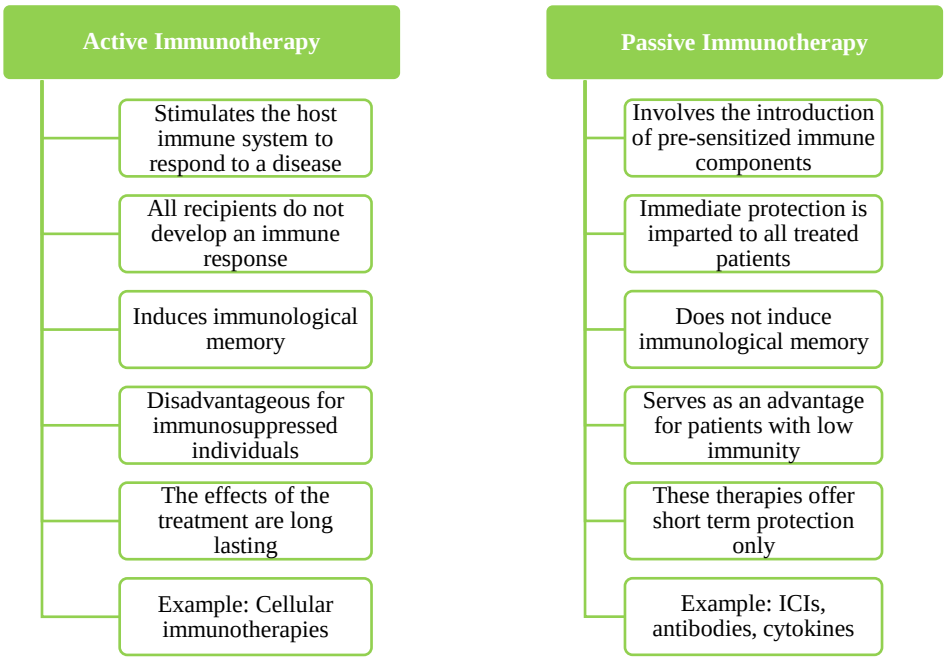
4.5.1.1. ACTIVE IMMUNOTHERAPY

Active immunotherapy aims to stimulate / activate the immune system using several methods, such as the administration of diseases-specific antigens to invoke an immune response. In case of cancers, the antigens present on tumor cells are used to train / stimulate the immune system to combat the specific disease. Once specific tumor antigens are recognized by the immune system, the body activates and mobilizes an army of lymphocytes and natural killer cells to detect and specifically eliminate the population of cells bearing the tumor antigen. However, this kind of a response is primarily governed by the presence of a unique antigen on the tumor cell that helps distinguish them from healthy cells. Examples of this approach include, dendritic cell based immunotherapy, T-cell based adoptive immunotherapy and therapeutic vaccines.[17, 18]

4.5.1.2. PASSIVE IMMUNOTHERAPY

Figure 4.2 outlines the major differences between the two classes of immunotherapy.

Figure 4.2 **Difference between Active and Passive Immunotherapies**



Source: RootRoots Analysis, http://www.sciencenutshell.com/difference-passive-active-immunotherapy-impact-cancer-treatment/

[17]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4971375/
[18]Source: http://www.sciencenutshell.com/difference-passive-active-immunotherapy-impact-cancer-treatment/

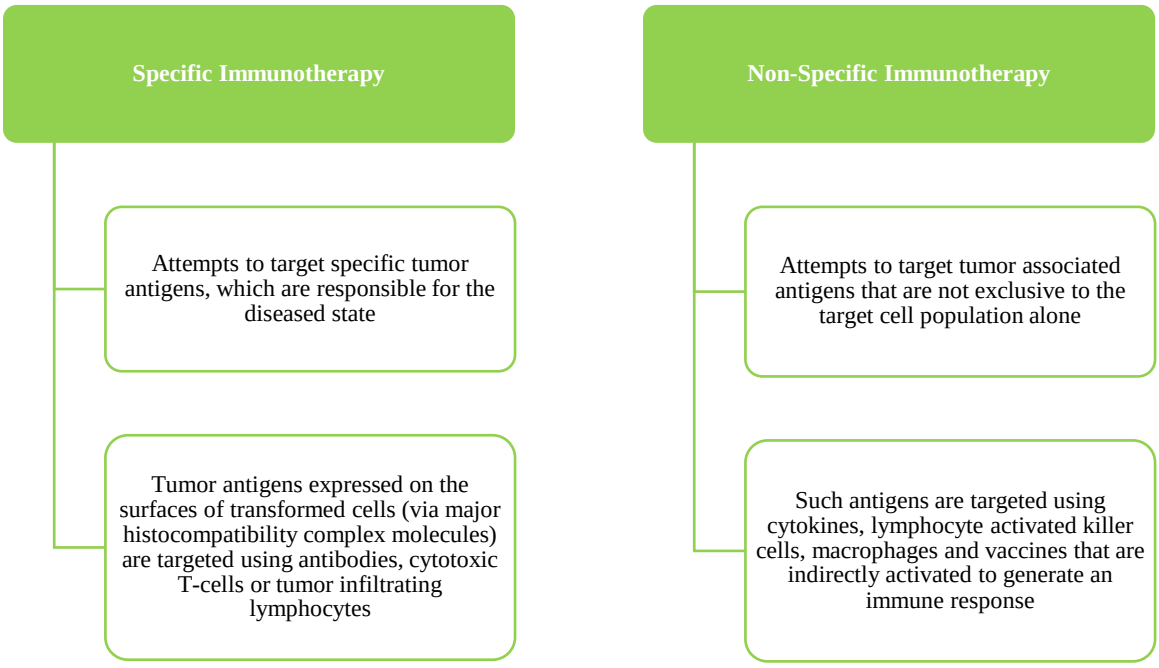
Passive immunotherapy involves the introduction of pre-sensitized immune system mediators, against a specific antigen, into a diseased host. These components may include one (or more) of several types of immune system mediators, such as antibodies, cytokines, T-cells and macrophages. This form of therapy provides immediate protection against the tumor antigens. Examples of immunosuppressive drugs based on this approach are cytostatic drugs, glucocorticoids and immunophilins.[19, 20]

4.5.2. By Type of Target

Immunotherapies can also be categorized as specific and non-specific based on the type of target.

Figure 4.3 highlights the key differences between specific and non-specific immunotherapies.

Figure 4.3 **Difference between Specific and Non-Specific Immunotherapies**



Source: Roots Analysis, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4350348/

4.5.3. By Approach

4.5.3.1. Activation and Suppression Immunotherapy

There are two main approaches that are used in immunotherapy, namely:

- Activation immunotherapy

[19]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4971375/
[20]Source: http://www.sciencenutshell.com/difference-passive-active-immunotherapy-impact-cancer-treatment/

- Suppression immunotherapy

Immunotherapies designed to elicit or amplify an immune response within a host are considered as **activation immunotherapies**. Examples of such therapies includeautologous immune enhancement therapy (AIET), dendritic cell based immunotherapy, T-cell based adoptive immunotherapy and therapeutic vaccines. Immunotherapies that are currently being developed for the treatment of cancer are mainly different types of activation immunotherapy.[21]

Immunotherapies designed to reduce or suppress an existing immune response, which is usually necessary in cases of autoimmunity or allergy, are classified as **suppression immunotherapies**. Examples of immunosuppressive drugs include cytostatic drugs, glucocorticoids and immunophilins.[22]

4.5.4. By Product Class

4.5.4.1. Monoclonal Antibodies

In this form of immunotherapy, patients are treated with monoclonal antibodies (mAbs), which are synthesized *invitro* and sensitized against tumor antigens. Currently, there are several mAb based therapies available in the market; examples include bevacizumab (Avastin[23]) for lung cancer, colon cancer and breast cancer, and trastuzumab (Herceptin[24]) for HER2+ breast cancer and HER2+ metastatic gastric cancer.[25, 26] Table 4.2 provides a list of all mAb based drugs that are approved in the US for the treatment of various oncological disorders.

Table 4.2 **FDA Approved Antibody Based Therapeutics for Cancer**

S. No.	Generic Name	Trade Name	Year of Approval in the US
1	Rituximab	MabThera[27], Rituxan[28]	1997
2	Trastuzumab	Herceptin	1998
3	Gemtuzumab ozogamicin	Mylotarg[29]	2000[30],2017
4	Alemtuzumab	MabCampath[31], Campath-1H	2001
5	Tositumomab-I131	Bexxar[32]	2003
6	Cetuximab	Erbitux[33]	2004

[21]Source: https://www.news-medical.net/health/Activation-Immunotherapies.aspx
[22]Source: https://www.news-medical.net/health/Suppression-Immunotherapies.aspx
[23] Avastin® is a registered trademark of Genentech(Roche)
[24] Herceptin® is a registered trademark of Genentech(Roche)
[25]Source: https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/monoclonal-antibodies.html
[26] Source: https://www.cancer.net/navigating-cancer-care/how-cancer-treated/immunotherapy-and-vaccines/understanding-immunotherapy
[27]MabThera® is a registered trademark of Genentech(Roche)
[28]Rituxan® is a registered trademark of Biogen Idec (Roche)
[29] Mylotarg® is a registered trademark of Pfizer
[30] This drug was initially approved in 2000, however,it was voluntarily withdrawn from the market. It was granted approval again in 2017
[31]MabCampath® is a registered trademark of Genzyme (Sanofi)
[32]Bexxar® is a registered trademark of Corixa
[33]Erbitux® is a registered trademark of Eli Lilly

S. No.	Generic Name	Trade Name	Year of Approval in the US
7	Ibritumomab tiuxetan	Zevalin[34]	2002
8	Bevacizumab	Avastin	2004
9	Panitumumab	Vectibix[35]	2006
10	Eculizumab	Soliris[36]	2007
11	Golimumab	Simponi[37]	2009
12	Tocilizumab	RoActemra, Actemra[38]	2010
13	Ofatumumab	Arzerra[39]	2009
14	Denosumab	Prolia[40]	2010
15	Ipilimumab	Yervoy[41]	2011
16	Brentuximab vedotin	Adcetris[42]	2011
17	Pertuzumab	Perjeta[43]	2012
18	Carfilzomib	Kyprolis[44]	2012
19	Trastuzumab emtansine	Kadcyla[45]	2013
20	Obinutuzumab	Gazyva[46]	2013
21	Ramucirumab	Cyramza[47]	2014
22	Siltuximab	Sylvant[48]	2014
23	Pembrolizumab	Keytruda[49]	2014
24	Blinatumomab	Blincyto[50]	2014
25	Nivolumab	Opdivo[51]	2014
26	Dinutuximab	Unituxin[52]	2015
27	Daratumumab	Darzalex[53]	2015
28	Idarucizumab	Praxbind[54]	2015
29	Elotuzumab	Empliciti[55]	2015
30	Necitumumab	Portrazza[56]	2015
31	Atezolizumab	Tecentriq[57]	2016
32	Olaratumab	Lartruvo[58]	2016

[34]Zevalin® is a registered trademark of Spectrum Pharmaceuticals
[35]Vectibix® is a registered trademark of Immunex Corporation (Amgen)
[36]Soliris® is a registered trademark of Alexion Pharmaceuticals
[37]Simponi® is a registered trademark of Johnson & Johnson
[38]Actemra® is a registered trademark of Chugai Seiyaku Kabushiki Kaisha
[39]Arzerra ® is a registered trademark of GSK
[40] Prolia® is a registered trademark of Amgen
[41] Yervoy® is a registered trademark of Bristol-Myers Squibb
[42] ADCETRIS® is a registered trademark of Seattle Genetics and Millennium Pharmaceuticals
[43] Perjeta® is a registered trademark of Genentech(Roche)
[44] Kyprolis® is a registered trademark of Onyx Pharmaceuticals
[45] Kadcyla® is a registered trademark of Genentech(Roche)
[46] Gazyva® is a registered trademarks of Genentech(Roche)
[47] Cyramza® is a registered trademark of Eli Lilly
[48]Sylvant® is a registered trademark of Johnson & Johnson
[49]Keytruda® is a registered trademark of Merck Sharp & Dohme
[50] Blincyto® is a registered trademark of Amgen
[51] Opdivo® is a registered trademark of Bristol-Myers Squibb
[52] Unituxin® is a registered trademark of United Therapeutics
[53] Darzalex® is a registered trademark of Johnson & Johnson
[54] Praxbind® is a registered trademark of Boehringer Ingelheim
[55] Empliciti® is a registered trademark of Bristol-Myers Squibb
[56] Portrazza™ is a trademark of Eli Lilly
[57] Tecentriq™ is a trademark of Genentech
[58] Lartruvo™ is a trademark of Eli Lilly

S. No.	Generic Name	Trade Name	Year of Approval in the US
34	Daclizumab	Zinbryta[59]	2016
35	Durvalumab	Imfinzi[60]	2017
36	Avelumab	Bavencio[61]	2017

Source: http://www.antibodysociety.org/news/approved_mabs.php

4.5.4.2. BISPECIFIC ANTIBODIES

A bispecific antibody (bsAb) is a second-generation immunotherapy, which represents an upgraded version of a monoclonal antibody with an improved structure and functionality. These are essentially antibodies that are synthesized by physically fusing two monoclonal antibodies or the specificity determining regions of two monoclonal antibodies. This results in the formation of chimeric immunoglobulin having different binding specificities that enable it to simultaneously bind to two different epitopes. Depending on the chosen target antigens, such a combination product may have additive or synergistic effects in combating a disease.

Over the years, various technology platforms, based on different antibody fusion concepts, such as chemical crosslinking, hybrid hybridomas and recombinant DNA techniques, have been developed and used for the production of bsAbs. Moreover, different binding sites can be exploited to produce bispecific systems with different mechanisms, such as the T-cell engager system.

Some of the advantages of bsAbs are provided below:[62]

- Since most diseases involve several parallel signaling pathways in the pathogenesis process, a therapeutic agent that can affect multiple pathways simultaneously is likely to be more efficient in treating the condition. bsAbs offer the advantage of being able to block / modify several tumors associated antigens instead of just one, as is the case with mAbs.
- Simultaneous targeting of multiple antigens limits the ability of tumor cells to evade the therapy. This also increases the tumor targeting specificity by delivering cytotoxic agents to tumor cells alone.
- Unlike conventional mAbs, bsAbs can be designed to direct T-cell mediated cytotoxicity. T-cells lack F_c receptors, due to which they are unable to bind to the F_c region of the mAbs. However, bsAbs can be designed to have a F_{ab} arm that is specific for the CD3 antigen of T-

[59]Zinbryta® is a registered trademark Biogen
[60] IMFINZI is a trademark of AstraZeneca
[61]BAVENCIO® is a registered trademark of Merck
[62]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4687327/

cells. Such a bsAb is capable of recruiting the activity of effector T-cells in the antitumor immune response that they elicit.

4.5.4.3. CYTOKINES

Cytokines are small proteins that are essential mediators of an immune response. Some cytokines that are currently being used for cancer treatment are described below:[63]

- Interleukins: Interleukins are known to stimulate or regulate immune cells. Studies have demonstrated that some patients suffering from metastatic melanoma can be completely cured with high-doses of interleukin-2 (IL-2) treatment alone, but most need such factors to be administered in combination with other treatments.[64] IL-2 is marketed under the brand name Proleukin[65]. It has also been shown to cause serious side effects, owing to which it can only be recommended to patients with healthy heart and lung function. Due to innate toxicity issues, IL-2 is not used much.
- Interferons: Interferons are chemicals produced in response to bacteria, viruses, and parasites. Interferon-α (IFN-α) has been shown to have direct impact on cancer cells, either to slow their growth or help revert them into the normal state.[66] IFN-α has been approved for the treatment of various types of cancer, including melanoma, leukemia and Kaposi's sarcoma. Some marketed products based on IFN-α include Roferon-A[67], Intron A[68] and Alferon N[69].

4.5.4.4. ONCOLYTIC VIRUS THERAPY

Oncolytic viruses are genetically engineered viruses that are designed to kill cancer cells. The anticancer activity of oncolytic viruses is due to the ability of these viruses to infect and replicate in the cancer cells. As a result of the viral copies, the cells burst and die. The release of specific antigens post cell lysis triggers the patient's immune system to target all the cancer cells in the body that have the same antigens. It is important to mention that these oncolytic viruses do not enter healthy cells. Imlygic[70] was the first oncolytic virus therapy to be approved by the FDA in October 2015for the treatment of melanoma. The virus used in the therapy is known as *Talimogene laherparepvec* or T-VEC. It is a genetically modified version of the *herpes simplex virus* that causes cold sores. T-VEC can be injected directly into areas of melanoma that a surgeon cannot remove.[71]

[63]Source: https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/nonspecific-immunotherapies.html
[64]Source: http://www.uptodate.com/contents/melanoma-treatment-advanced-or-metastatic-melanoma-beyond-the-basics
[65]Proleukin® is a registered trademark of Prometheus Laboratories
[66]Source: https://www.medicinenet.com/interferon/article.htm
[67]Roferon® A is a registered trademark of Roche
[68]Intron® A is a registered trademark of Schering-Plough
[69]Alferon™ is a trademark of Hemispherx Biopharma
[70] IMLYGIC® is a registered trademark of Amgen
[71]Source: https://www.cancer.net/navigating-cancer-care/how-cancer-treated/immunotherapy-and-vaccines/understanding-immunotherapy

4.5.4.5. THERAPEUTIC CANCER VACCINES

Therapeutic vaccines are a viable option to treat late stage cancer by using a patient's immune system. Unlike traditional preventative vaccines that are generally administered to healthy individuals, therapeutic cancer vaccines are administrated to cancer patients and are designed to eradicate cancer cells by strengthening the patient's immune responses against tumor antigens; they are intended to delay or stop the growth of advanced tumors and / or relapsed tumors that are refractory to standard of care therapies. Therapeutic cancer vaccines consist of a recombinant protein containing tumor antigen and an immune cell activator. Once the vaccine is injected, the antigen is processed and expressed by the APCs, which interact with T-cells to initiate a T-cell drive immune response. Cancer vaccines are of two types; autologous tumor cell vaccines (derived from the patient's tumor cells) and allogeneic tumor cell vaccines (derived from established human tumor cell lines). Examples of some the cancer vaccines approved by the FDA include Gardasil[72], Gardasil 9[73] and Cervarix[74].[75, 76]

4.5.4.6. CELL BASED THERAPIES

This approach involves the use of either autologous or allogenic whole cells that may or may not be modified, to invoke an immune response to fight a disease. The most common types of cell based therapies are discussed below:

- **Dendritic Cell Therapy:** This involves the isolation of a patient's own APCs, which are then loaded with tumor specific antigen(s) and re-introduced back into the patient's body. This potentiates an enhanced immune response against cells bearing the specific tumor antigen(s).[77] In April 2010, the very first immunostimulant therapy, Sipuleucel-T (Provenge[78]), was approved. Sipuleucel-T is a dendritic cell vaccine that was developed and manufactured by Dendreon.[79]
- **Chimeric Antigen Receptor T-cells (CAR-T):** This novel cancer therapy platform involves isolation of T-cells in an approach that is similar to that used in dendritic cell therapy. Autologous T-cells are modified to express a synthetic receptor that enables them to recognize tumor specific antigens and mount an immune response against cells bearing such antigens. Initial efforts in this domain were focused on the development of a CAR-T therapy against the CD19 receptor found on B-cells.[80]

[72] GARDASIL® is a registered trademark of Merck
[73]GARDASIL 9® is a registered trademark of Merck
[74] CERVARIX® is a registered trademark of GlaxoSmithKline
[75]Source: http://www.fightcancerwithimmunotherapy.com/Portals/1/PDF/ONC_Monograph_Revised_CY_4_4_Final3.pdf
[76]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3721379/
[77]Source: https://www.canceractive.com/cancer-active-page-link.aspx?n=3080
[78]Provenge® is a registered trademark of Dendreon
[79]Source: http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/ucm210012.htm
[80]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3183449/

- **T-Cell Receptors (TCRs):** TCRs are a complex of integral membrane proteins that are exclusively found on T-cells. They are essentially responsible for antigen recognitionand their subsequent activation. Such therapies are currently under clinical studies for the treatment of several hematological malignancies and solid tumors.[81]
- **Tumor Infiltrating Lymphocytes (TILs):** It is a preparation of autologous engineered lymphocytes. Therapeutic TILs are derived from tumor tissue and are amplified by *in vitro* culturing in the presence of various lymphokines, such as IL-2, which are known to augment the cytotoxic activity of these cells. Upon administration, these lymphocytes infiltrate the tumor, and induce tumor regression and tumor cells lysis. There is sufficient clinical evidence supporting the efficacy of TILs in treating patients with advanced cancers.[82]

4.6. HISTORICAL EVOLUTIONOF T-CELL IMMUNOTHERAPIES

Lymphocytes are critical components of the immune system and play a major role in both innate and adaptive immune responses.[83] Within cells that originate from the lymphoid lineage, T-lymphocytes or T-cells are considered to be one of the primary mediators of adaptive immunity and are essential for immunosurveillance. In addition, these cells have been shown to be involved in tumor suppression and in preventing the malignant transformation of normal cells into a cancerous phenotype.

Adoptive immunotherapy is an emerging concept that involves the passive transfer of immune cells, which may or may not be modified / genetically altered to express desired traits and / or features. These cells may be tailored to treat different type of diseases and malignancies, such as cancers and viral infections. In such therapies, immune cells are either obtained from the patients themselves (autologous) or from healthy donors (allogeneic). These cells are then sensitized to specific disease antigen(s) and amplified *invitro* before being infused into the host. Once inside the body of the patient, the modified lymphocytes are capable of recognizing target cells bearing the antigen that they are sensitized to and selectively eliminating them, thereby, combating the disease in a specific and controlled manner.[84]

The history of adoptive immunotherapy can be traced back to the 1980s, when lymphokine-activated killer (LAK) cells were first used to treat cancer in mice and humans. This was

[81]Source: https://www.thermofisher.com/in/en/home/life-science/cell-analysis/signaling-pathways/t-cell-receptor-tcr/t-cell-receptor-tcr-overview.html
[82]Source: https://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=41004
[83]Source: http://missinglink.ucsf.edu/lm/immunology_module/prologue/objectives/obj02.html
[84]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4327320/

followed by the evaluation of cytokine-induced killer (CIK) cells, isolated from peripheral blood mononuclear cells (PBMCs), to treat cancer in the 1990s. However, LAK and CIK-based therapies demonstrated limited efficacy due to the lack of specificity. In the early 1990s, adoptive cell therapy was first described by Dr. Phil Greenberg[85] and his team at the Fred Hutchinson Cancer Research Center(FHCRC). They demonstrated how T-cells could be extracted, expanded exponentially and infused back into a diseased human body to provide therapeutic benefit. Later, such therapies were developed as a treatment option for cancer indications, such as melanoma, prostate cancer and aggressive leukemia. In 1988, TILs became the first T-cell based therapy to be used for the treatment of melanoma. Later, it was found that lymphodepletion (destruction of lymphocytes and T-cells) before autologous TIL infusion achieved objective anticancer responses ranging from 49% to 72%. This is because lymphodepletion helps in the depletion of suppressive cells, such as Treg cells, in the tumor micro-environment and in the blood. This enables the survival and multiplication of adoptively transferred TILs to achieve effective killing of cancer cells. TIL-based therapy has been shown to lead to long-term remission of more than five years and low recurrence rate in the treated patients. However, successful implementation of TIL therapies is associated with several limitations, including surgical removal of tissues from tumors for culturing TILs. Moreover, there are only few medical centers worldwide that offer TIL based therapy due to the fact that TIL isolation and culturing requires highly skilled medical personnel. In order to overcome these barriers, genetically engineered T-cells and unmodified peptide-stimulated T-cells, such as CAR-T cells and TCR cells, that target specific antigen expressed on cancer cells have been employed in clinical trials and have demonstrated promising and exciting results.[86, 87]

The use of T-cells as therapeutic candidates is supported by the following characteristics:[88]

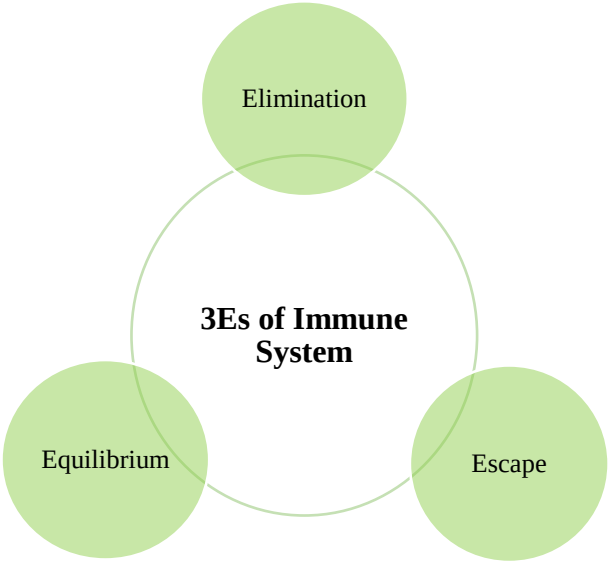
- **Target specificity:** As mentioned earlier, T-cells are capable of specifically targeting cells that bear the antigen(s) against which a particular population of cells is sensitized. For this purpose, identification of specific molecular targets is crucial to channelizing an immune response in the most effective and efficient manner. An advantage offered by this characteristic is the prevention of on-target off-tumor toxicity.
- **Cellular trafficking:** These cells are capable of collective migration towards the target cancer cell population for executing an immune response. This mobility of T- cells across the body is crucial to the execution of a targeted immune response.

[85] Dr. Phil Greenberg is the Head of Immunology at Fred Hutchinson Cancer Research Center
[86]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4372895/
[87]Source:https://www.fredhutch.org/en/diseases/featured-researchers/greenberg-philip.html
[88]Source: http://drfarrahcancercenter.com/cancer-immunotherapy-the-fundamentals/#

- **Adaptability:** T-cells are able to adapt to the surrounding microenvironment and carry out their designated functions at the target site. Processes such as epitope spreading and antigen spreading convene this step of the immune response.
- **Memory:** This is considered to be one of the most important features of the T-cell mediated immune response. As mentioned earlier, T-cells are capable of retaining immunological memory. This refers to the ability of these cells to recognize disease specific antigens and mount an effective immune response even after a long period of time from the initial encounter.

Figure 4.4 outlines the 3Es of the immune system that explain the dynamic relationship between immune mediators and cancer cells.

Figure 4.4 **3Es of the Immune System**



Source: Roots Analysis, http://www.fightcancerwithimmunotherapy.com/Portals/1/PDF/ONC_Monograph_Revised_CY_4_4_Final3.pdf

During the elimination phase, cancer cells are recognized and effectively eliminated from the body. This is immediately followed by the equilibrium state. It is at this point that the body is capable of preventing any further growth of the cancerous cells. It is worth mentioning that, in such a state, cancerous cells persist but are inhibited from multiplying. This balanced phase is followed by the escape phase, where the immune system is overwhelmed by the disease and fails to further control the expansion of the cancer cell population. This state eventually leads to the onset of progressive disease.[89]

[89]Source: http://www.fightcancerwithimmunotherapy.com/Portals/1/PDF/ONC_Monograph_Revised_CY_4_4_Final3.pdf

A better understanding of this domain has led to the identification of certain distinct states that are now known as the *Hallmarks of Cancer*. An article published a few years ago by Hanahan and Weinberg summarizes the aforementioned hallmarks. The key insights from the article are outlined below:[90]

- Cancerous cells are capable of growing in the absence of growth signals.
- Cancerous cells are able to circumvent programs that negatively regulate cell proliferation; in other words, they have the mechanism to evade growth suppressors. Cancerous cells are able to induce the formation of support structures in their microenvironment that assist in their proliferation.
- These cells are capable of spreading across the entire body, thereby, weakening the host's defense mechanisms and eventually leading to the death of an individual.
- The metabolism of normal cells is significantly hampered due to the uncontrolled proliferation of transformed cells.
- Cancerous cells overwhelm the immune system and result in malignant disease.

4.7. KEY CONSIDERATIONS OF T-CELL IMMUNOTHERAPIES

The use of T-cells for therapeutic purposes is dependent on a number of factors. One of the primary enabling factors behind such therapy candidates are reprogramming technologies, such as genetic alterations and structural modifications, which are used to confer enhanced targeting and stability characteristics to naïve T-cells.

The key considerations while reprogramming / engineering T-cells, for development of targeted therapies, in order to invoke a substantial immune response are listed below:

- Selection of an antigenic target
- Generation of a T-cell response, having high avidity and high magnitude
- Stimulation of T-cell infiltration at the site of cancerous growth
- Facilitate the development of immunological memory

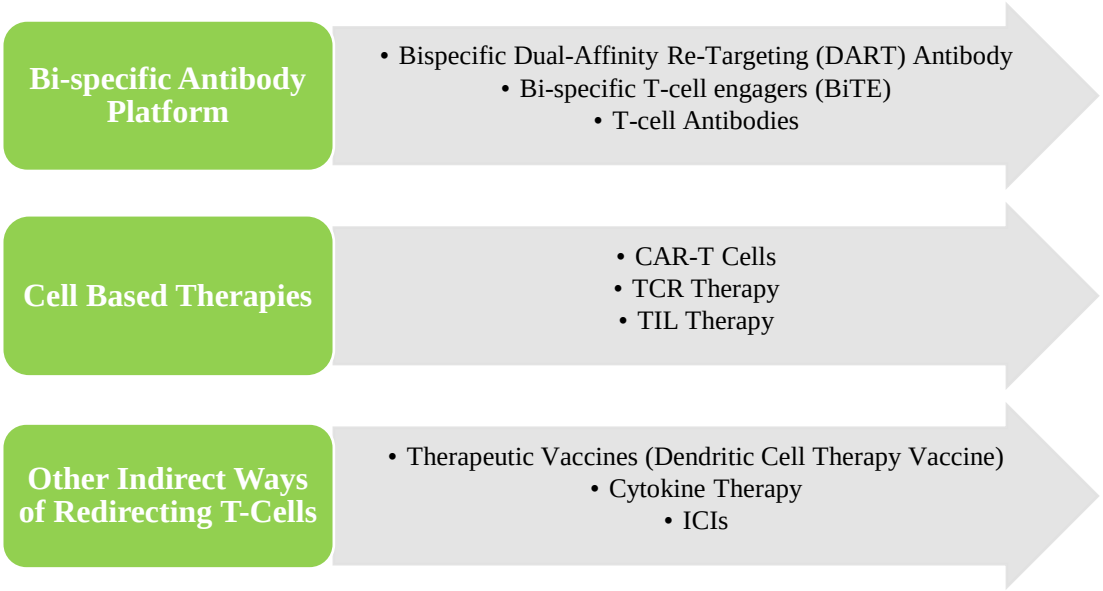
4.8. STRATEGIES EMPLOYED FOR REDIRECTION OF T-CELLS

Advances in molecular research and genetic manipulation techniques have led to the development of several platforms that can be used to harness the underlying potentials of T-cells and direct their functionality to offer therapeutic benefit.

[90]Source: http://www.sciencedirect.com/science/article/pii/S0092867411001279

Figure 4.5 outlines the various strategies that are used to redirect T-cells in order to elicit the desired immune response.

Figure 4.5 **Strategies Employed for the Redirection of T-Cells**



Source: Roots Analysis

The scope of this report is limited to the three cell-based therapies mentioned in the figure, namely:

- CAR-T cell therapy
- TCR based therapy
- TIL based therapy

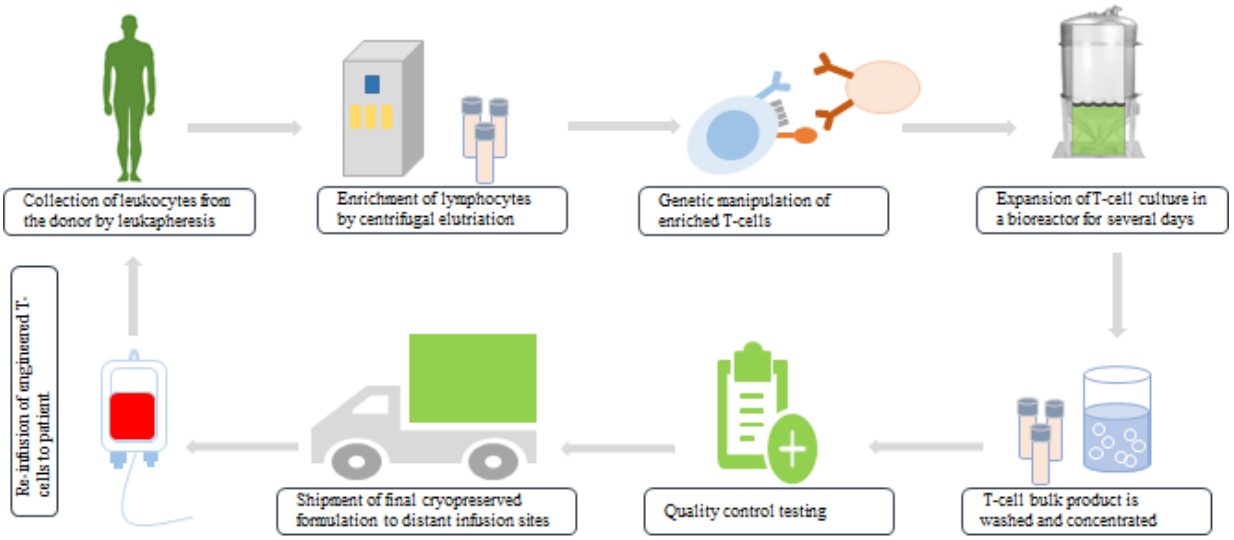
Additionally, the report includes brief descriptions of other novel T-cell based immunotherapies that are being developed by various industry players. These details are presented in Chapter 14.

4.9. Manufacturing of Engineered T-Cells

Manufacturing of T-cell therapy begins with the collection of leukocytes from the patient's blood by leukapheresis. The collected leukocytes are further enriched by using counterflow centrifugal elutriation or subsets selection. The genetic information of the enriched leukocytes is then manipulated by culturing them with bead-based artificial APCs, followed by addition of viral vectors. For several days, the culture is expanded in the bioreactor. Post amplification, engineered T-cells are washed and concentrated. Samples are taken from this bulk product to conduct quality control testing. The final product is cryopreserved and is shipped to distant infusion sites, where the product bag is thawed and infused back to the patient. Generally,

production of T-cell therapies takes 5 to 10 days. The total time period from collection of lymphocytes to infusion may take around 2-4 weeks depending on the clinical status of the patient and chemotherapy conditioning regimens.[91] Figure 4.6 represents the general manufacturing process of T-cell therapies.

Figure4.6 **T-Cell Manufacturing: General Procedure**



Source: Roots Analysis, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5543811/figure/F3/

4.10. T-CELL TRANSDUCTION / TRANSFECTION METHODS

The manipulation of cells at the genetic level is often required to program them to function in a desired manner. Such manipulations generally involve the introduction of transgenes. Several advances in biotechnology have led to the development of efficient gene transfer mechanisms that also facilitate the expression of a variety of human genes in target cells. Gene delivery into target cells is facilitated by vectors. Over the last few years, various viral and non-viral vectors have been developed and optimized for this purpose. The use of viral vectors for gene transfer is known as transduction, while non-viral modes of gene transfer are referred to as transfection.[92] Of all available methods, the most commonly used vectors for introducing novel genes into T-cells are retrovirus and lentivirus based vectors.[93, 94] These two viral systems, as well as non-viral transfection methods are discussed in the following sections.

4.10.1. RETROVIRAL VECTORS

Retroviruses were the first viruses to be used as gene therapy vectors. They are RNA viruses that belong to the *Retroviridae* family. These viruses are capable of synthesizing double

[91]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5543811/figure/F3/
[92]Source: https://www.sciencedirect.com/science/article/pii/S0960894X1500030X
[93]Source: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0056027
[94]Source: http://info.evaluategroup.com/rs/607-YGS-364/images/epv-cart16.pdf

stranded DNA copies of their RNA genomes using an enzyme known as reverse transcriptase. These DNA copies can be integrated into the chromosome of host cell by an integrase enzyme carried by the virus. Stable integration of DNA copies of the viral genome modifies the host cell, which synthesizes viral proteins along with host proteins. When the modified host cell divides, daughter cells retain the new genes.[95] Table 4.3 summarizes the salient features of retroviral vectors.

Table 4.3 **Retroviral Vectors: Salient Features**

Parameters	Description
Genetic Material	ssRNA
Coat	Enveloped
Tropism	Dividing cells only
Host Genome Interaction	Integrating
Transgene Expression	Stable (long-lasting)
Packaging Capacity	8 kb (8,000 base pairs)
Inflammatory Potential	Low
Advantages	▪ Stable gene expression ▪ Reasonable space to accommodate therapeutic gene post removal of non-essential viral genes ▪ Retroviral promoter can direct efficient expression of transgenes encoded within its genome ▪ No / very low pre-existing immunity ▪ Vector particles produced in high titers (10^6-10^8 pfu/ml)
Disadvantages	▪ Only transduces dividing cells ▪ Insertional mutagenesis results in unwanted mutations, which may lead to the development of tumors ▪ Recombination events may lead to the production of wild-type viruses

Pfu: Plaque forming units

Source: http://www.ohsu.edu/xd/about/services/integrity/upload/IBC_Presentation-Choosing-a-Viral-Vector-System.pdf

4.10.2. LENTIVIRAL VECTORS

Lentiviruses are RNA viruses that also belong to the *Retroviridae* family. Similar to retroviruses, lentiviruses are capable of inserting the therapeutic gene into the genome of the host cell (stable gene expression). However, unlike retroviruses, lentiviral vectors can infect non-dividing cells as well. The only cells that lentiviruses cannot gain access to are quiescent cells (those that are in the G_0 state of the cell cycle).[96, 97, 98] This is primarily because these cells block the reverse transcription step of the viral infection process. Examples of some lentivirus are listed below:

- Human immunodeficiency virus (HIV)
- Simian immunodeficiency virus (SIV)

[95]Source: http://www.genetherapynet.com/viral-vector/retroviruses.html
[96]Source: http://www.genetherapynet.com/viral-vector/lentiviruses.html
[97]Source: http://www.ohsu.edu/xd/about/services/integrity/upload/IBC_Presentation-Choosing-a-Viral-Vector-System.pdf
[98]Source: https://www.proprofs.com/discuss/q/773528/what-cell-types-can-lentiviral-vectors-infect-check-all-that

- Feline immunodeficiency virus (FIV)
- Equine infectious anemia virus (EIAV)

Lentiviral vectors have been used in the development of therapies for various disease indications, including adrenoleukodystrophy, Wiskott-Aldrich syndrome, various cancers, Parkinson's disease and retinitis pigmentosa. Although there isn't any *invivo* or *invitro* data supporting the presence / generation of replication competent lentiviral strains during the therapy development or treatment, there are still concerns related to the possibility of occurrence of the same. Therefore, in order to prevent any lethal human infections due to reversion to wild type strains, vectors based on non-human lentiviral species, such as FIV, SIV and EIAV, are being used. Table 4.4 summarizes the salient features of lentiviral vectors.

Table 4.4 **Lentiviral Vectors: Salient Features**

Parameters	Description
Genetic Material	ssRNA
Coat	Enveloped
Tropism	Dividing and non-dividing cells
Host Genome Interaction	Integrating
Transgene Expression	Stable (long-lasting)
Packaging Capacity	8 kb (8,000 base pairs)
Inflammatory Potential	Low
Advantages	▪ Stable gene expression ▪ High-efficiency infection of dividing and non-dividing cells
Disadvantages	▪ Recombination to produce wild-type virus

Source: http://www.genetherapynet.com/viral-vectors.html, http://www.ohsu.edu/xd/about/services/integrity/upload/IBC_Presentation-Choosing-a-Viral-Vector-System.pdf

4.10.3. NON-VIRAL TRANSFECTION METHODS

Although retrovirus and lentivirus based vectors are commonly used for introducing novel genes into T-cells, they are typically associated with high manufacturing costs, safety concerns (insertional mutagenesis, immunogenicity) and restrictions on genetic payload. Due to these limitations, non-viral transfection methods, such as electroporation techniques, nanoparticles, liposomal formulation and cell-penetrating peptides, are widely being adopted to transfer to novel genetic material into T-cells as these are associated with low risk of immunogenicity and insertional mutagenesis. Particularly, non-viral electroporation methods allow the delivery of larger gene inserts and are more cost-effective as compared to viral methods. Some early results even suggest that CAR-T cells produced by using non-viral electroporation methods are effective in treating certain types of cancer, such as tyrosine kinase inhibitors resistant Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL). Considering these

advantages, non-viral transfection via electroporation is likely to become one of the preferred methods over viral-mediated transduction for engineering CAR T-cells in the future. However, non-viral transfection methods are associated with certain limitations related to their clinical application in human cancer therapy. It has been observed that due to the low efficiency of gene transfer and subsequent insufficient integration into the T-cell genome, non-viral methods are difficult to validate in human applications. However, combination of DNA transposition methods with enhanced electroporation techniques has shown great potential in resolving these challenges.[99]

Amongst the non-viral systems used for the delivery of transgenes in T-cell therapy, transposon-transposase systems, such as fish-derived Sleeping Beauty (SB) and insect-derived piggyBac human-adapted transposition systems, have emerged as preferred methods to generate safe, inexpensive and effective therapeutic CAR-T cells. In addition to non-viral transposition systems, other new systems, such as transiently expressed mRNAs, are being investigated to reduce the unwanted toxicity of genetically modified T-cells. These transiently expressed mRNAs are being used to control CAR expression so that it can be switched on or off to limit on-target, off-tissue toxicity. However, the limitation of such technique is that it cannot provide long-term expression that is required for maximal CAR-T cell function and sustained defense against cancer.

More recently, clustered regularly interspaced short palindromic repeats (CRISPR) / Cas9 has been employed to deliver CAR sequences into T-cells. The technology allows the targeted integration of CAR sequences into the TCR locus, enabling endogenous control of CAR expression with parallel knockout of the TCR. This results in generation of safer and more effective therapeutic CAR- T cells. Additionally, CRISPR / Cas9 has been employed to knockout the inhibitory checkpoint PD-1 receptor in T-cells to enhance the efficiency of T-cell based therapeutics. Several studies have indicated the potential of CRISPR / Cas9 genome editing in enhancing of the safety and efficacy of immunotherapies.[100]

4.10.3.1. SLEEPING BEAUTY TRANSPOSON

There are two major shortcomings associated with non-viral gene therapy approaches, which are related to the delivery of genes using engineered plasmids that are produced in *E. coli* and their expression.[101]However, the Sleeping Beauty Transposon System (SBTS) is known to

[99]Source: https://www.biosciencetechnology.com/article/2017/08/vital-role-emerging-gene-transfer-methods-t-cell-cancer-therapy
[100]Source: https://www.biosciencetechnology.com/article/2017/08/vital-role-emerging-gene-transfer-methods-t-cell-cancer-therapy
[101]Source: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3095056/#DDR140C23

overcome these concerns by combining the key features of viral vectors and naked DNA vectors.

The SBTS comprises of two major components, namely a transposon, which contains a gene expressing cassette, and a transposase enzyme. Several studies have demonstrated the advantages of this system. For example, SBTS has been successfully used to enable the sustained expression of α1-antitrypsin in normal mice and clotting factor (FIX) in the hemophilic mice. It has also been used for the successful treatment of various other diseases, such as epidermolysis bullosa, glioblastoma multiform, sickle cell anemia and B-cell lymphoma.

One of the first clinical trials that used the SBTS was conducted in patients suffering from leukemia and lymphoma.[102] Safety and feasibility results of the transposon system were presented at the 55th American Society of Hematology (ASH) annual meeting and exposition. According to the results of the study, no instances of acute or late toxicities were reported during the first five months post administration of the therapy. Some of the prominent advantages of the SBTS include simple, faster and nimble process for customizing T-cells.[103]

When compared to existing transposon-based gene transfer systems, the SBTS has been proven to be more efficient, specifically for applications in human gene therapy. Some of the salient features of the SBTS include high efficacy, ease of delivery (methods and routes) and safety. The system is also cost effective. On the other hand, certain disadvantages, such as the lack of efficient delivery tools and inability of maintaining the gene expression in the liver, also exist and need to be addressed.

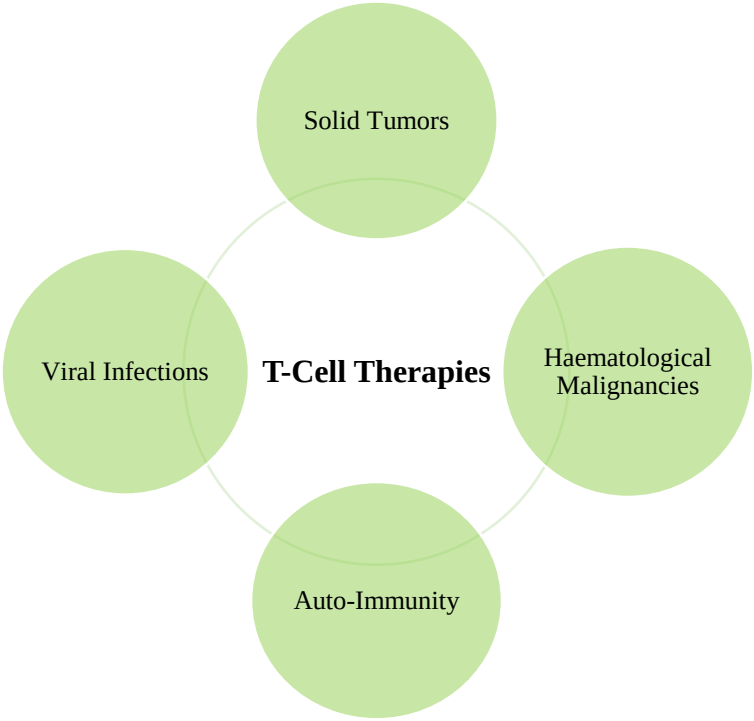
4.11. T-CELL IMMUNOTHERAPY: TARGETED THERAPEUTIC AREAS

T-cell based therapies are being widely tested across metastatic melanoma and several other hematological cancers. Marked by effective and significant clinical results, the T-cell immunotherapy market is evolving at a commendable pace. In addition, therapy developers are exploring the potential of these therapies to target other therapeutic areas, such as infectious diseases and autoimmune diseases.[104]

[102]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5004935/
[103]Source: http://www.cancerfrontline.org/researchers_call_on_sleeping_beauty_to_arm_t_cells_against_cancer_ash13/
[104]Source: https://www.nature.com/articles/nature22395

Further, a number of technology developers have made significant contributions to this domain in terms of the establishment of novel cell therapy development platforms and gene modification procedures. The development of molecular switches and associated technological platforms have significantly impacted the market providing the necessary capabilities to address the major gaps in prevalent therapies. Immunotherapies are highly customizable and therefore, possess the potential to treat a variety of different therapeutic indications. Figure 4.7 highlights the key therapeutic areas being targeted by T-cell based therapies.

Figure 4.7 **T-Cell Immunotherapy: Targeted Therapeutic Areas**



Source: Roots Analysis

4.12. T-CELL IMMUNOTHERAPIES: KEY CHALLENGES

Besides the various advantages offered by T-cell based therapies, there are certain issues that have restricted the pace of growth in the market. Some of the prominent challenges have been discussed below:[105]

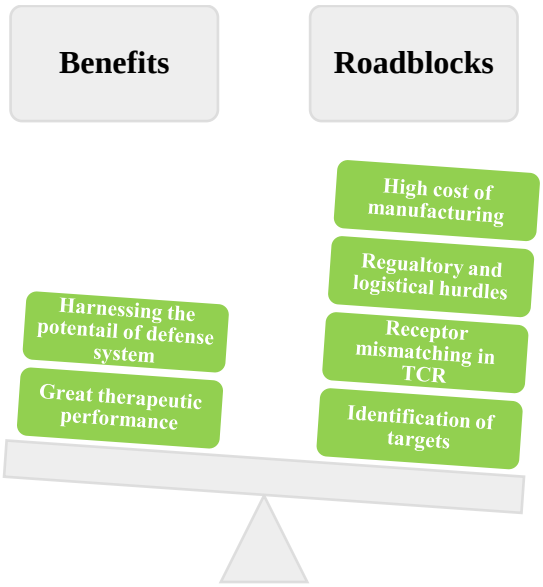
- The identification of target antigens is quite a tedious task, and needs significant laboratory and technical expertise.
- Maintaining the quality T-cells post *invitro* expansion is expected to be a constraint in cases of negligence and when inadequate methodologies are employed for such purposes
- Regulatory and logistical hurdles related to these therapies are prohibitive in most cases

[105]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4381333/

- Receptor mismatching in TCRs has been shown to lead to several adverse events
- Lethal on-target off-tumor toxicity has also been reported in many cases where such therapies have been used. Many of these issues have already been addressed; however, certain areas of concern continue to persist
- The cost intensive nature of the manufacturing process is another big challenge to this industry

Figure 4.8 presents a comparative representation of the benefits and challenges associated with the T-cell immunotherapy market.

Figure 4.8 T-Cell Immunotherapies: Benefits and Key Challenges



Source: Roots Analysis

However, both industry and non-industry players are currently engaged in extensive research to develop technologies to overcome these roadblocks. Over the past few years, immunotherapy has been successful in carving out a significant niche in the pharmaceutical space. As indicated before, several such targeted therapies have already been approved and a robust development pipeline indicates that the immunotherapy market is likely to grow at a steady pace in the foreseen future. Various factors, such as successful preclinical results, lucrative funding opportunities and expedited development / review provisions facilitating the speedy approval of such products, have emerged as some of the key drivers in this domain.

5

5. SOCIAL MEDIA ANALYSIS

5.1. CHAPTER OVERVIEW

Over the last few years, online social media platforms have brought about a paradigm shift in communication, expression of views and advertising. Widespread access to social media platforms has brought several novel innovations, which would have otherwise gone unnoticed earlier, into the limelight. The vast potential of social media as a public relations tool, as well as a marketing tool, has motivated a number of stakeholders in the biopharmaceutical sector to become active on such online platforms. Eminent leaders and key players in this industry share insights on their work and keep their followers up to date on all their initiatives. In addition, distributors and consumers are also free to express their opinions regarding a particular product on such platforms. Therefore, tracking the activity of the pharmaceutical industry on social media often provides valuable insights that cannot be ignored.

As mentioned earlier, pharma companies use such channels for marketing their products and connecting to customers / patients / physicians. In addition to sharing press releases and clinical trial results, certain companies are also known to have established patient support groups and online communities to help those who may not otherwise consult a healthcare service provider due to various reasons.

Twitter is one of the most popular online platforms wherein thousands of individuals follow recent activities in pharmaceutical / biopharmaceutical industry. In this chapter, we have presented an analysis of the prevalent trends related to T-cell immunotherapies as represented on Twitter.

5.2. TRENDS ON TWITTER

5.2.1. T-CELL IMMUNOTHERAPIES: YEARLY TRENDS ON TWITTER

Twitter is considered to be the third most popular social media platform till date.[106] For this analysis, we downloaded tweets from the period between January 2012 and April 2019. The tweets contained various keywords related to T-cell immunotherapies and information related to various clinical trials, partnerships and factors responsible for popularity of this domain. Additionally, the tweets showcase the most significant events responsible for increase in the volume of tweets each year.

Figure 5.1 provides an overview of the downloaded tweets based on the keywords *T-Cell Immunotherapy, CAR-T Therapy, TCR Therapy* and *TIL Therapy*,on Twitter.

Figure 5.1**T-Cell Immunotherapy Social Media Analysis: Twitter Trends, January 2012-April2019**

	UserName	FullName	Date	Quarter	First Hash	Alt Hashta	Tweet (M	Type of Immunothe	Therapy Name	Retweets	Retweets	Likes	Likes (w/	URL Path	Check
2	@cure_tal	Priya Men	30-Nov-17	Q2 2017			Discussions on recently FDA approved CAR-T Cell			1 1 1 1	Retweet	1	Like	/cure_talk	#N/A
3	@WCHFou	WCH Four	30-Nov-17	Q2 2017			Organising Host, Mike Garrick, captured rallying 9			3 3	Retweet		Like	/WCHFou	#N/A
4	@doug_m	Doug McB	30-Nov-17	Q2 2017			If I was terminal I would want the latest tech yes. I			1	Retweet		Like	/doug_mc	#N/A
5	@ASGCTh	ASGCTâ€Â	30-Nov-17	Q2 2017	#CART,Cel	#CellTher	Breakthrough therapy designation granted to #CA			1 1	Retweet		Like	/ASGCThe	#N/A
6	@cells_nr	Alexey Be	1-Dec-17	Q2 2017			$KITE formed relationships with GE "to develop a			3 9 9 14 14	Retweet	9	Like	/cells_nnr	#N/A
7	@matthew	Matthew I	1-Dec-17	Q2 2017			And one last round of thanks to the patients who			1 1 1 4 4	Retweet	1	Like	/matthew	#N/A
8	@TheSkep	The Skept	1-Dec-17	Q2 2017	#caseclosed		Last night I asked Arie Belldegrun if he considered			1 1 1 5 5	Retweet	1	Like	/TheSkep	#N/A
9	@PCF_Sci	PCF Scien	1-Dec-17	Q2 2017	#immunotherapy		Thank you @fabianaperna for sharing your excelle			2 2	Retweet		Like	/PCF_Scie	#N/A
10	@Aifa_uff	AIFAâ€Â @	1-Dec-17	Q2 2017	#medicina		.@mmelazzini "innovazione sta cambiando volto			1 3 3 2 2	Retweet	3	Like	/Aifa_uffi	#N/A
11	@nilogen	Nilogen O	1-Dec-17	Q2 2017			CAR-T cells get an A for 2017! http://ow.ly/BQPV30gPZDy				Retweet		Like	/nilogeno	#N/A
12	@susandu	Susan Dur	1-Dec-17	Q2 2017	#immunotherapy		#immunotherapy Are Viruses a Problem for CAR-T			1 1 1 1	Retweet	1	Like	/susandur	#N/A
13	@_B_I_O_	Gene Edit	1-Dec-17	Q2 2017	#Biotech,#	#gene	Jefferies says: #Biotech $XBI $IBB $NBI Due for a B			3 17 17 35	Retweet	17	Like	/_B_I_O_T	#N/A
14	@cure_tal	Priya Men	1-Dec-17	Q2 2017	#Chemoth	#newinca	How is CAR-T Cell Therapy Different from Standar			1 1 1 1	Retweet	1	Like	/cure_talk	#N/A
15	@OxfordO	OLPâ€Â @	1-Dec-17	Q2 2017			"An â€œalmost miraculousâ€ therapy for some ch			1 1	Retweet		Like	/OxfordOl	#N/A
16	@TongMe	é€šå,³åª'â	1-Dec-17	Q2 2017			ã€é,å'å…¨çƒç¬¬ä¸€æ¯ éšŠï¼ä¸-åœ‹ç™Œç—‡è¨ºæ²»ç™‚,åƒ'a				Retweet		Like	/TongMec	#N/A
17	@LLSusa	The Leuke	4-Dec-17	Q2 2017	#leukemia		Cheering on this incredible 7-year-old survivor, w			7 7 31 31	Retweet	7	Like	/LLSusa/st	#N/A
18	@adamfe	Adam Feu	5-Dec-17	Q2 2017			$CELG $BLUE signal quick start to CAR-T trial in mu			1 30 30 49	Retweet	30	Like	/adamfeu	#N/A
19	@joesvill	Joe Redm	5-Dec-17	Q2 2017			Update on Joe. It seems that Joeâ€™s CAR T-Cells			23 10 10 6(	Retweet	10	Like	/joesvilla	#N/A
20	@CIRMne	CIRMâ€Ve	5-Dec-17	Q2 2017	#clinicaltr	#cancer	Poseida Therapeutics advances preclinical CAR-T s			1 5 5 11 11	Retweet	5	Like	/CIRMnew	#N/A
21	@Mohty_	Mohamad	6-Dec-17	Q2 2017	#ASH17		A few food for thoughts during #ASH17 @TheEBM			13 13 17 1	Retweet	13	Like	/Mohty_E	#N/A
22	@cells_nr	Alexey Be	6-Dec-17	Q2 2017			$JUNO to acquire a license to the GSI from Eli Lilly			1 6 6 10 10	Retweet	6	Like	/cells_nnr	#N/A
23	@bradlon	Brad Lonc	6-Dec-17	Q2 2017			Exactly one year ago at this time we were just see			3 9 9 42 42	Retweet	9	Like	/bradlonc	#N/A
24	@Vincent	Vincent Ra	6-Dec-17	Q2 2017	#ASH17,A	#ASH17VR	Iâ€™d rather cost-effectiveness studies be done b			2 16 16 27	Retweet	16	Like	/VincentR	#N/A
25	@JorgeCo	Jorge Con	7-Dec-17	Q2 2017			With engineered cells (CAR-T), we entered the er			2 10 10 32	Retweet	10	Like	/JorgeCon	#N/A
26	@_B_I_O_	Gene Edit	7-Dec-17	Q2 2017	#gene,edi	#editing	Jefferies: $GILD Small acquisition confirmed to ma			2 7 7 20 20	Retweet	7	Like	/_B_I_O_T	#N/A

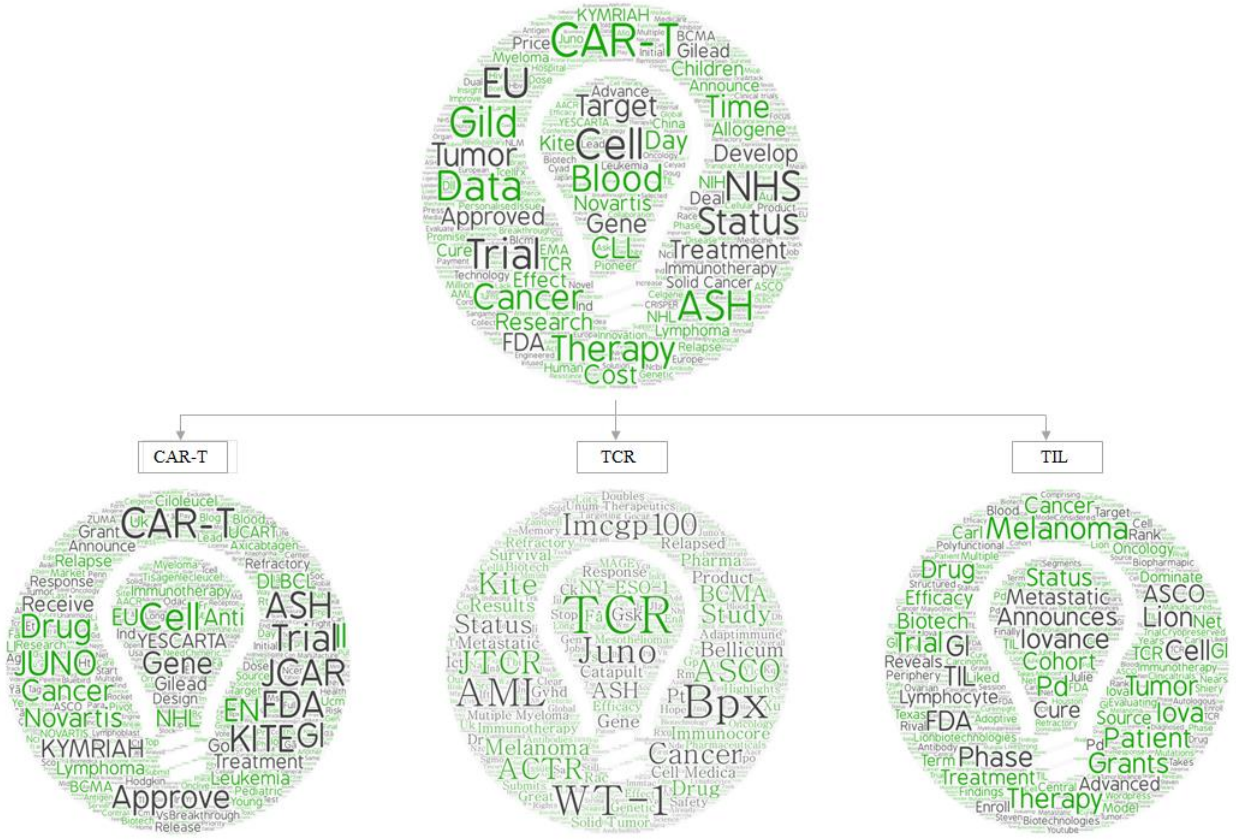
Source: Roots Analysis, www.twitter.com

5.2.2. T-CELL IMMUNOTHERAPIES: POPULAR KEYWORDS ON TWITTER

Figure 5.2 provides additional insights on the tweets containing the keywords *T-Cell Immunotherapy, CAR-T Therapy, TCR Therapy* and *TIL Therapy*,along with the tweets captured using the names of the marketed or clinical product candidates being developed by industrial players.

[106]Facebook and YouTube are the first two most popular social media platforms based upon the estimated unique monthly visitors http://www.ebizmba.com/articles/social-networking-websites

Figure 5.2 **T-Cell Immunotherapy Social Media Analysis: Popular Keywords on Twitter, January 2012-April 2019**



Note: Commonly used words, such as prepositions, conjunctions, articles and internet jargon (www, .com and google) have been removed

Source: https://tagul.com/, www.twitter.com, Roots Analysis

In the figure, the top most word cloud was generated using data from all the tweets that we came across. Further, we performed separate word cloud analyses on tweets that mentioned either of the three major classes of T-cell immunotherapies, namely CAR-T, TCR and TIL. As indicated in the figure, C*AR-T, Approve, Kite, Juno, Novartis, Kymriah* and *Yescarta*emerged as some of the most frequently used words in association with the keyword(s) used for this analysis. In addition, we observed that CAR-T appeared to be the most popular word (*in terms of the frequency of appearance in the sample dataset*) used with reference to this type of therapy. This was followed by TCR and TIL therapies.

Detailed analyses of other parameters have been included in the project report. However, it cannot be revealed due to confidentiality purposes.

6

6. T-CELL IMMUNOTHERAPIES: PIPELINE

A comprehensive research is carried out to build T-cell Immunotherapy pipeline where multiple parameters of each of these therapieswere captured. The pipeline consisted of close to 650 therapies providing information on several parameters, such as:

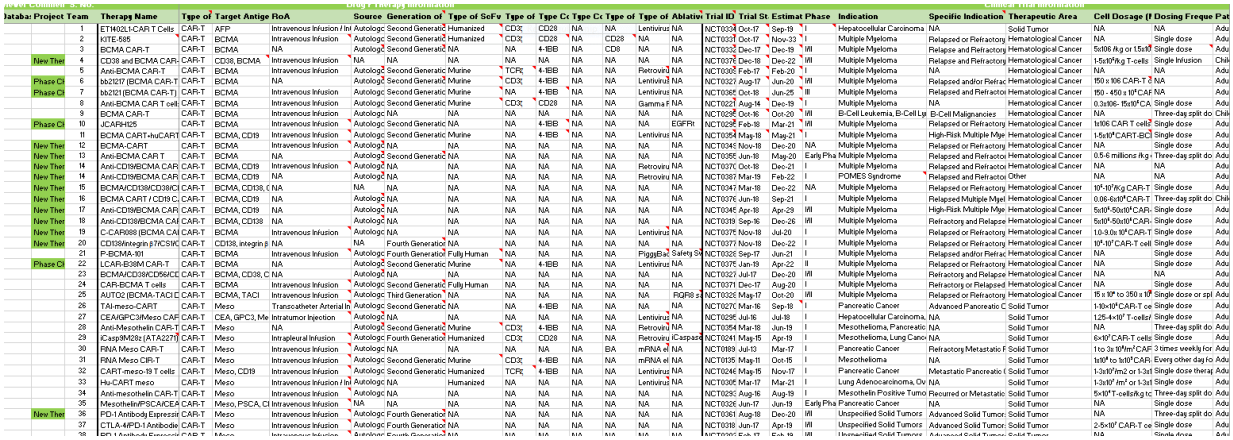
- Drug / Therapy Information
  - Therapy Name
  - Type of Product
  - Target Antigen
  - Route of Administration
  - Source of T-Cells
  - Designation
  - Generation of CAR-T
  - Type of ScFv
  - Type of Signaling Domain
  - Type Co-Stimulatory Domain 1
  - Type Co-Stimulatory Domain 2
  - Type of Transmembrane domain
  - Type of Vector
  - Ablative technology, if any
- Clinical Trial Information
  - Trial ID
  - Trial Start Date
  - Estimated Trial End Date
  - Phase
  - Indication
  - Specific Indication
  - Therapeutic Area
  - Cell Dosage (No. of T-cells Administered)
  - Dosing Frequency

- Patient Segment
- Type of Therapy

- Developer Information
  - Sponsor
  - Collaborators
  - Type of Developers
  -

Figure 6.1, 6.2 and 6.3 provides a glimpse of the pipeline and various parameters captured.

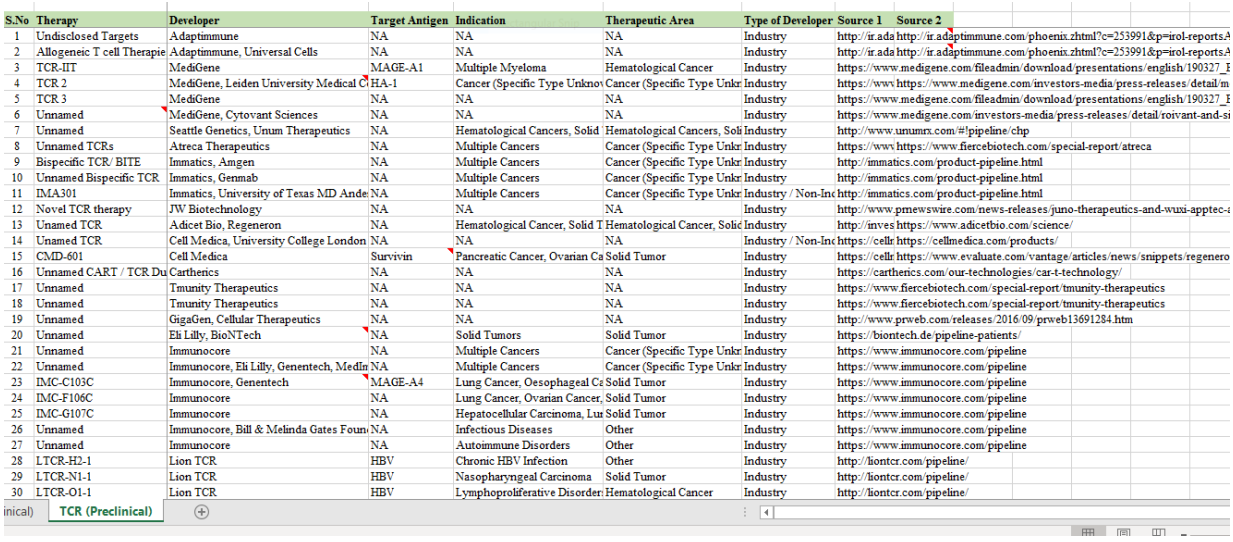
Figure 6.1 **CAR-T Pipeline Glimpse 1**



	Therapy Name	Type of	Target Antige	RoA	Source	Generation of	Type of Sc	Type of	Type Co	Type Co	Type of	Type of	Ablativ	Trial ID	Trial St	Estimat	Phase	Indication	Specific Indication	Therapeutic Area	Cell Dosage (P	Dosing Freque	Pat
1	ET402L1-CAR T Cells	CAR-T	AFP	Intravenous Infusion / Int	Autologc	Second Generatic	Humanized	CD3ζ	CD28	NA	NA	Lentivirus	NA	NCT0334	Oct-17	Sep-19	I	Hepatocellular Carcinoma	NA	Solid Tumor	NA	NA	Adu
2	KITE-585	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	Humanized	CD3ζ	CD28	NA	CD28	NA	NA	NCT0333	Oct-17	Nov-33	I	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	NA	Single dose	Adu
3	BCMA CAR-T	CAR-T	BCMA	NA	Autologc	Second Generatic	NA	NA	4-1BB	NA	CD8	NA	NA	NCT0332	Dec-17	Dec-19	I/II	Multiple Myeloma	Relapse and Refractory	Hematological Cancer	5x106 /kg or 15x10	Single dose	Adu
New Ther 4	CD38 and BCMA CAR-	CAR-T	CD38, BCMA	Intravenous Infusion	NA	NA	NA	NA	NA	NA	NA	NA	NA	NCT0327	Dec-18	Dec-22	I/II	Multiple Myeloma	Relapse and Refractory	Hematological Cancer	1-5x10⁶/kg T-cells	Single Infusion	Chil
5	Anti-BCMA CAR-T	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	Murine	TCRζ	4-1BB	NA	NA	Retrovira	NA	NCT0326	Feb-17	Feb-20	I	Multiple Myeloma	NA	Hematological Cancer	NA	NA	Adu
Phase Cl 6	bb21217 (BCMA CAR-T	CAR-T	BCMA	NA	Autologc	Second Generatic	Murine	CD3ζ	4-1BB	NA	NA	Lentivirus	NA	NCT0322	Aug-17	Jun-20	I/II	Multiple Myeloma	Relapsed and/or Refrac	Hematological Cancer	150 x 106 CAR-T c	NA	Adu
Phase Cl 7	bb2121 (BCMA CAR-T)	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	Murine	NA	4-1BB	NA	NA	Lentivirus	NA	NCT0305	Oct-16	Jun-25	III	Multiple Myeloma	Relapsed and Refractor	Hematological Cancer	150 - 450 x 10⁶ CAR	NA	Adu
8	Anti-BCMA CAR T cell	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	Murine	CD3ζ	CD28	NA	NA	Gamma R	NA	NCT0322	Aug-14	Dec-19	I	Multiple Myeloma	NA	Hematological Cancer	0.2x106- 15x10⁶CA	Single dose	Adu
9	BCMA CAR-T	CAR-T	BCMA	Intravenous Infusion	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0329	Oct-16	Oct-20	I/II	B-Cell Leukemia, B-Cell Ly	B-Cell Malignancies	Hematological Cancer	NA	Three-day split do	Chil
Phase Cl 10	JCARH125	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	NA	4-1BB	NA	NA	NA	EGFRt	NCT0329	Feb-18	Mar-21	I/II	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	1x106 CAR T cells	Single dose	Adu	
11	BCMA CART+huCART	CAR-T	BCMA, CD19	Intravenous Infusion	Autologc	Second Generatic	Murine	4-1BB	NA	NA	Lentivirus	NA	NCT0304	May-18	May-21	I	Multiple Myeloma	High-Risk Multiple Mye	Hematological Cancer	1-5x10⁶ CART-BC	Single dose	Adu	
New Ther 12	BCMA-CART	CAR-T	BCMA	Intravenous Infusion	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0304	Nov-18	Dec-20	NA	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	NA	Single dose	Adu
New Ther 13	Anti-BCMA CAR T	CAR-T	BCMA	NA	Autologc	Second Generatic	NA	NA	NA	NA	NA	NA	NA	NCT0305	Jun-18	May-20	Early Pha	Multiple Myeloma	Relapsed and Refractor	Hematological Cancer	0.5-6 millions /kg	Three-day split do	Adu
New Ther 14	Anti-CD19/BCMA CAR	CAR-T	BCMA, CD19	Intravenous Infusion	Autologc	NA	NA	NA	NA	NA	NA	Retrovira	NA	NCT0307	Oct-18	Dec-21	I	Multiple Myeloma	Relapsed and Refractor	Hematological Cancer	NA	NA	Adu
New Ther 14	Anti-CD19/BCMA CAR	CAR-T	BCMA, CD19	NA	Autologc	NA	NA	NA	NA	NA	NA	Retrovira	NA	NCT0307	Mar-19	Feb-22	I	POMES Syndrome	Relapsed and Refractor	Other	NA	NA	Adu
New Ther 15	BCMA/CD19/CD38/CI	CAR-T	BCMA, CD38, C	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NCT0304	Mar-18	Dec-22	NA	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	10⁶-10⁷/Kg CAR-T	Single dose	Adu
New Ther 16	BCMA CART / CD19 C	CAR-T	BCMA, CD19	NA	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0307	Jun-18	Sep-21	I	Multiple Myeloma	Relapsed Multiple Myel	Hematological Cancer	0.08-6x10⁶CAR-T	Three-day split do	Chil
New Ther 17	Anti-CD19/BCMA CAR	CAR-T	BCMA, CD19	NA	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0304	Apr-18	Apr-29	I/II	Multiple Myeloma	High-Risk Multiple Mye	Hematological Cancer	5x10⁶-50x10⁶CAR-	Single dose	Adu
New Ther 18	Anti-CD138/BCMA CA	CAR-T	BCMA, CD138	NA	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0301	Sep-16	Dec-26	I/II	Multiple Myeloma	Refractory and Relapse	Hematological Cancer	5x10⁶-50x10⁶CAR-	Single dose	Adu
New Ther 19	C-CAR088 (BCMA CA	CAR-T	BCMA	Intravenous Infusion	Autologc	NA	NA	NA	NA	NA	NA	Lentivirus	NA	NCT0307	Nov-18	Jul-20	I	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	1.0-9.0x 10⁶ CAR-T	Single dose	Adu
New Ther 20	CD19/integrin β7/CS1(	CAR-T	CD19, integrin β	NA	NA	Fourth Generatic	NA	NA	NA	NA	NA	NA	NA	NCT0377	Nov-18	Dec-22	I	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	10⁶-10⁷ CAR-T cell	Single dose	Adu
21	P-BCMA-101	CAR-T	BCMA	Intravenous Infusion	Autologc	Fourth Generatic	Fully Human	NA	NA	NA	NA	PiggyBac	Safety St	NCT0328	Sep-17	Jun-21	I	Multiple Myeloma	Relapsed and/or Refrac	Hematological Cancer	NA	Single dose	Adu
Phase Cl 22	LCAR-B38M CAR-T	CAR-T	BCMA	NA	Autologc	Second Generatic	Murine	NA	4-1BB	NA	NA	Lentivirus	NA	NCT0375	Jan-19	Apr-22	II	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	NA	Single dose	Adu
23	BCMA/CD38/CD56/CD	CAR-T	BCMA, CD38, C	NA	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0327	Jul-17	Dec-20	I/II	Multiple Myeloma	Refractory and Relapse	Hematological Cancer	NA	NA	Adu
24	CAR-BCMA T cells	CAR-T	BCMA	Intravenous Infusion	Autologc	Second Generatic	Fully Human	NA	NA	NA	NA	NA	NA	NCT0377	Dec-17	Aug-20	I	Multiple Myeloma	Refractory or Relapsed	Hematological Cancer	NA	Single dose	Adu
25	AUTO2 (BCMA-TACI C	CAR-T	BCMA, TACI	Intravenous Infusion	Autologc	Third Generation	NA	NA	NA	NA	RQR8 a	NCT0302	May-17	Oct-20	I/II	Multiple Myeloma	Relapsed or Refractory	Hematological Cancer	15 x 10⁶ to 350 x 10	Single dose or spl	Adu		
26	TAI-meso-CART	CAR-T	Meso	Transcatheter Arterial In	Autologc	Second Generatic	NA	4-1BB	NA	NA	NA	NA	NCT0270	Mar-16	Sep-18	I	Pancreatic Cancer	Advanced Pancreatic C	Solid Tumor	1-10x10⁶ CAR-T ce	Single dose	Adu	
27	CEA/GPC3/Meso CAR	CAR-T	CEA, GPC3, Me	Intratumor Injection	Autologc	NA	NA	NA	NA	NA	Lentivirus	NA	NCT0295	Jul-16	Jul-18	I	Hepatocellular Carcinoma,	NA	Solid Tumor	1.25-4x10⁷ T-cells/	Single dose	Adu	
28	Anti-Mesothelin CAR-T	CAR-T	Meso	NA	Autologc	Second Generatic	4-1BB	NA	NA	NA	Retrovira	NCT0305	Mar-18	Jun-19	I	Mesothelioma, Pancreatic	NA	Solid Tumor	NA	Three-day split do	Adu		
29	(Carg9M28z (ATA2271	CAR-T	Meso	Intrapleural Infusion	Autologc	Fourth Generatic	Humanized	CD3ζ	CD28	NA	NA	Retrovira	(Carg9M28z	NCT0324	May-15	Apr-19	I	Mesothelioma, Lung Canc	NA	Solid Tumor	6x10⁷ CAR-T cells	Single dose	Adu	
30	RNA Meso CAR-T	CAR-T	Meso	Intravenous Infusion	Autologc	NA	NA	NA	NA	BA	mRNA el	NA	NCT0189	Jul-13	Mar-17	I	Pancreatic Cancer	Refractory Metastatic F	Solid Tumor	1 to 3 x 10⁹/m² CAR	3 times weekly for	Adu	
31	RNA Meso CIR-T	CAR-T	Meso	Intravenous Infusion	Autologc	Second Generatic	Murine	CD3ζ	4-1BB	NA	NA	mRNA el	NA	NCT0135	May-11	Oct-15	I	Mesothelioma	NA	Solid Tumor	1x10⁸ to 1x10⁹ CAR-	Every other day fo	Adu
32	CART-meso-19 T cells	CAR-T	Meso, CD19	Intravenous Infusion	Autologc	Second Generatic	Humanized	TCRζ	4-1BB	NA	NA	Lentivirus	NA	NCT0324	May-15	Nov-17	I	Pancreatic Cancer	Metastatic Pancreatic (	Solid Tumor	1-3x10⁶/m2 or 1-3x1	Single dose therap	Adu
33	Hu-CART-meso	CAR-T	Meso	Intravenous Infusion / Int	Autologc	NA	Humanized	NA	NA	NA	NA	Lentivirus	NA	NCT0305	Mar-17	Mar-21	I	Lung Adenocarcinoma, Ov	NA	Solid Tumor	1-3x10⁷ /m² or 1-3x1	Single dose	Adu
34	Anti-mesothelin CAR-T	CAR-T	Meso	Intravenous Infusion	Autologc	NA	NA	NA	NA	NA	NA	NA	NA	NCT0292	Aug-16	Aug-19	I	Mesothelin Positive Tumo	Recurred or Metastatic	Solid Tumor	5x10⁶ T-cells/kg tc	Three-day split do	Adu
35	MesothelinVPSCA/CEA	CAR-T	Meso, PSCA, CI	Intravenous Infusion	NA	NA	NA	NA	NA	NA	NA	NA	NA	NCT0326	Jun-17	Jun-19	Early Pha	Pancreatic Cancer	NA	Solid Tumor	NA	Single dose	Adu
New Ther 36	PD-1 Antibody Expressi	CAR-T	Meso	Intravenous Infusion	Autologc	Fourth Generatic	NA	NA	NA	NA	NA	NA	NA	NCT0361	Aug-18	Dec-20	I/II	Unspecified Solid Tumors	Advanced Solid Tumor	Solid Tumor	NA	Three-day split do	Adu
37	CTLA-4/PD-1 Antibodie	CAR-T	Meso	Intravenous Infusion	Autologc	Fourth Generatic	NA	NA	NA	NA	NA	NA	NA	NCT0318	Jun-17	Apr-19	I/II	Unspecified Solid Tumors	Advanced Solid Tumor	Solid Tumor	2.5x10⁷ CAR-T ce	Single dose	Adu

Source: Roots Analysis

Figure 6.2 **TCR Pipeline Glimpse 2**



S.No	Therapy	Developer	Target Antigen	Indication	Therapeutic Area	Type of Developer	Source 1	Source 2
1	Undisclosed Targets	Adaptimmune	NA	NA	NA	Industry	http://ir.ada	http://ir.adaptimmune.com/phoenix.zhtml?c=253991&p=irol-reports/
2	Allogeneic T cell Therapie	Adaptimmune, Universal Cells	NA	NA	NA	Industry	http://ir.ada	http://ir.adaptimmune.com/phoenix.zhtml?c=253991&p=irol-reports/
3	TCR-IIT	MediGene	MAGE-A1	Multiple Myeloma	Hematological Cancer	Industry	https://www.medigene.com/fileadmin/download/presentations/english/190327_I	
4	TCR 2	MediGene, Leiden University Medical C	HA-1	Cancer (Specific Type Unkno	Cancer (Specific Type Unkr	Industry	https://ww	https://www.medigene.com/investors-media/press-releases/detail/m
5	TCR 3	MediGene	NA	NA	NA	Industry	https://www.medigene.com/fileadmin/download/presentations/english/190327_I	
6	Unnamed	MediGene, Cytovant Sciences	NA	NA	NA	Industry	https://www.medigene.com/investors-media/press-releases/detail/roivant-and-si	
7	Unnamed	Seattle Genetics, Unum Therapeutics	NA	Hematological Cancers, Solid	Hematological Cancers, Soli	Industry	http://www.unumrx.com/#!pipeline/chp	
8	Unnamed TCRs	Atreca Therapeutics	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry	https://ww	https://www.fiercebiotech.com/special-report/atreca
9	Bispecific TCR/ BITE	Immatics, Amgen	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry	http://immatics.com/product-pipeline.html	
10	Unnamed Bispecific TCR	Immatics, Genmab	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry	http://immatics.com/product-pipeline.html	
11	IMA301	Immatics, University of Texas MD Ande	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry / Non-Ind	http://immatics.com/product-pipeline.html	
12	Novel TCR therapy	JW Biotechnology	NA	NA	NA	Industry	http://www.prnewswire.com/news-releases/juno-therapeutics-and-wuxi-apptec-a	
13	Unamed TCR	Adicet Bio, Regeneron	NA	Hematological Cancer, Solid T	Hematological Cancer, Solid	Industry	http://inves	https://www.adicetbio.com/science/
14	Unamed TCR	Cell Medica, University College London	NA	NA	NA	Industry / Non-Ind	https://cellr	https://cellmedica.com/products/
15	CMD-601	Cell Medica	Survivin	Pancreatic Cancer, Ovarian Ca	Solid Tumor	Industry	https://cellr	https://www.evaluate.com/vantage/articles/news/snippets/regenero
16	Unnamed CART / TCR Du	Cartherics	NA	NA	NA	Industry	https://cartherics.com/our-technologies/car-t-technology/	
17	Unnamed	Tmunity Therapeutics	NA	NA	NA	Industry	https://www.fiercebiotech.com/special-report/tmunity-therapeutics	
18	Unnamed	Tmunity Therapeutics	NA	NA	NA	Industry	https://www.fiercebiotech.com/special-report/tmunity-therapeutics	
19	Unnamed	GigaGen, Cellular Therapeutics	NA	NA	NA	Industry	http://www.prweb.com/releases/2016/09/prweb13691284.htm	
20	Unnamed	Eli Lilly, BioNTech	NA	Solid Tumors	Solid Tumor	Industry	https://biontech.de/pipeline-patients/	
21	Unnamed	Immunocore	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry	https://www.immunocore.com/pipeline	
22	Unnamed	Immunocore, Eli Lilly, Genentech, Medin	NA	Multiple Cancers	Cancer (Specific Type Unkr	Industry	https://www.immunocore.com/pipeline	
23	IMC-C103C	Immunocore, Genentech	MAGE-A4	Lung Cancer, Oesophageal Ca	Solid Tumor	Industry	https://www.immunocore.com/pipeline	
24	IMC-F106C	Immunocore	NA	Lung Cancer, Ovarian Cancer,	Solid Tumor	Industry	https://www.immunocore.com/pipeline	
25	IMC-G107C	Immunocore	NA	Hepatocellular Carcinoma, Lur	Solid Tumor	Industry	https://www.immunocore.com/pipeline	
26	Unnamed	Immunocore, Bill & Melinda Gates Foun	NA	Infectious Diseases	Other	Industry	https://www.immunocore.com/pipeline	
27	Unnamed	Immunocore	NA	Autoimmune Disorders	Other	Industry	https://www.immunocore.com/pipeline	
28	LTCR-H2-1	Lion TCR	HBV	Chronic HBV Infection	Other	Industry	http://liontcr.com/pipeline/	
29	LTCR-N1-1	Lion TCR	HBV	Nasopharyngeal Carcinoma	Solid Tumor	Industry	http://liontcr.com/pipeline/	
30	LTCR-O1-1	Lion TCR	HBV	Lymphoproliferative Disorder	Hematological Cancer	Industry	http://liontcr.com/pipeline/	

inical) TCR (Preclinical)

Source: Roots Analysis

Figure 6.3 **TIL Pipeline Glimpse 3**

Project Team		Therapy Name	Type of T	RoA	Source of T-Cells	Trial ID	Trial Sta	Estimate	Phase	Indication	Specific Indi	Therapeutic A	Cell Dos	Dosing Freq	Patient Se	Type of Therapy	Sponsor	Collabor	Type of Developer
	1	TILs	TIL	NA	NA	NCT00200577	May-05	Mar-12	III	Melanoma	NA	Solid Tumor	NA	Two injections	Child, Adult,	Combination Therap	Nantes Univers	NA	Non-Industry
New Therapy	2	TILs	TIL	NA	NA	NCT03374839	Feb-18	Mar-21	III	Melanoma	Metastatic Mela	Solid Tumor	$0.5x10^{9}$ TIL	Two injections	Adult, Senior	Combination Therap	Nantes Univers	Bristol-My	Industry / Non-Industry
	3	TILs	TIL	NA	NA	NCT02278887	Sep-14	Sep-20	III	Melanoma	Stage IV Melan	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	The Netherlan	Copenhag	Non-Industry
	4	Lifileucel (LN-144)	TIL	NA	Autologous T-Cells	NCT02360579	Sep-15	Dec-24	II	Melanoma	Metastatic Mela	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Iovance Biothe	NA	Industry
New Indicatio	5	LN-145	TIL	Intravenous Infusion	Autologous T-Cells	NCT03645328	Mar-19	Dec-24	II	Melanoma, Squan	NA	Solid Tumor	NA	Single dose	Adult, Senior	Combination Therap	Iovance Biothe	NA	Industry
	5	LN-145	TIL	Intravenous Infusion	Autologous T-Cells	NCT03108495	Jun-17	Jun-24	II	Cervical Carcinom	Recurrent, Met	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Iovance Biothe	NA	Industry
New Indicatio	5	LN-145	TIL	Intravenous Infusion	Autologous T-Cells	NCT03449108	Apr-18	Dec-20	II	Soft Tissue Sarcor	Recurrent Soft	Solid Tumor	NA	Single dose	Child, Adult,	Combination Therap	Iovance Biothe	MD Ander	Industry / Non-Industry
New Therapy	6	MDA-TIL	TIL	Intravenous Infusion	Autologous T-Cells	NCT03610490	Aug-18	Sep-21	II	Ovarian Cancer, F	NA	Solid Tumor	NA	Single dose	Child, Adult,	Combination Therap	Iovance Biothe	MD Ander	Industry / Non-Industry
	7	Young TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01585428	Apr-12	Aug-16	II	Cervical Cancer, C	NA	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	National Canc	Iovance B	Industry / Non-Industry
	7	Young TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01174121	Aug-10	Dec-24	II	Colorectal Cancer	NA	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	National Canc	Iovance B	Industry / Non-Industry
	7	Young TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01993719	Dec-13	Sep-29	II	Melanoma	Metastatic Mela	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	National Canc	Iovance B	Industry / Non-Industry
	8	TILs	TIL	NA	Autologous T-Cells	NCT02421640	Mar-15	Mar-20	II	Nasopharyngeal C	NA	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Sun Yat-sen U	NA	Non-Industry
New Indicatio	8	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT03904537	Feb-19	Dec-21	III	Colorectal Cancer	Stage III Colore	Solid Tumor	0.1 to $1x10^{1}$	Single dose	Adult, Senior	Combination Therap	Sun Yat-sen U	NA	Non-Industry
New Indicatio	8	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT03903887	Feb-19	Dec-21	III	Non-Small Cell Lu	Stage II-IIIA Nor	Solid Tumor	0.1 to $1x10^{1}$	Single dose	Adult, Senior	Combination Therap	Sun Yat-sen U	NA	Non-Industry
	8	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01462903	Sep-11	Dec-14	I	Hepatocellular Ca	NA	Solid Tumor	10^{9} TILs to	NA	Adult, Senior	Combination Therap	Sun Yat-sen U	NA	Non-Industry
	9	TILs	TIL	Intravenous Infusion	NA	NCT02500576	Aug-15	Aug-20	II	Melanoma	Metastatic Mela	Solid Tumor	NA	Single dose	Adult, Senior	Combination Therap	MD Anderson (	Merck, Pro	Industry / Non-Industry
	10	TILs	TIL	Intravenous Infusion	NA	NCT00338377	Feb-06	Feb-19	II	Melanoma	Metastatic Mela	Solid Tumor	$1.5x10^{11}$ TIL	Single dose	Child, Adult,	Combination Therap	MD Anderson (	Prometheu	Industry / Non-Industry
	11	TILs	TIL	Intravenous Infusion	NA	NCT01740557	Jan-15	Jan-21	III	Melanoma	Metastatic Mela	Solid Tumor	$1.5x10^{11}$ TIL	Single dose	Adult, Senior	Combination Therap	MD Anderson (	National In	Industry / Non-Industry
	12	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01955460	Oct-14	Oct-19	I	Melanoma	Metastatic Mela	Solid Tumor	$1.5x10^{11}$ TIL	Single dose	Child, Adult,	Combination Therap	MD Anderson (	Cancer Pr	Non-Industry
	13	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01883323	Jun-13	Apr-18	II	Melanoma	Metastatic, Sta	Solid Tumor	$1x10^{11}$ TILs	Single dose	Adult, Senior	Combination Therap	University Heal	NA	Non-Industry
	13	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT02414945	Jun-15	Jun-25	III	Pleural Mesothelio	Malignant Pleu	Solid Tumor	$1x10^{11}$ TILs	Single dose	Adult, Senior	Combination Therap	University Heal	NA	Non-Industry
	14	Re-Stimulated TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01883297	Jan-15	Dec-23	I	Ovarian Cancer, F	High Grade Ser	Solid Tumor	$3x10^{11}$ TILs	Single dose	Adult, Senior	Combination Therap	University Heal	NA	Non-Industry
	15	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT03158935	Jul-17	Jun-20	I	Ovarian Cancer, M	Advanced Ova	Solid Tumor	$1x10^{11}$ TILs	Single dose	Adult, Senior	Combination Therap	University Heal	Merck	Industry / Non-Industry
	16	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01807182	Jun-13	Jun-19	II	Melanoma	Metastatic Mela	Solid Tumor	NA	Single dose	Adult, Senior	Combination Therap	Fred Hutchinso	National C	Non-Industry
New Indicatio	17	Donor Lymphocytes	TIL	Intravenous Infusion	Allogenic T-Cells	NCT03537599	Feb-19	Sep-21	III	Acute Myeloid Leu	Relapsed Acut	Hematological C	NA	NA	Child, Adult,	Combination Therap	National Canc	NA	Non-Industry
	17	Donor Lymphocytes	TIL	NA	Allogenic T-Cells	NCT01445132	Jan-07	Apr-13	I	Chronic Lymphocy	NA	Hematological C	$1.0x10^{6}$ TIL	NA	Adult, Senior	Monotherapy	National Canc	NA	Non-Industry
	18	TILs	TIL	NA	Autologous T-Cells	NCT03166397	Jun-17	Jun-21	II	Melanoma	Metastatic Mela	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Sheba Medica	NA	Non-Industry
New Indicatio	18	TILs	TIL	NA	Autologous T-Cells	NCT03412526	Jan-18	Feb-22	II	Ovarian Cancer	Metastatic Ova	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Sheba Medica	NA	Non-Industry
	19	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT02354690	Nov-14	Dec-18	III	Melanoma	Metastatic Mela	Solid Tumor	10^{9} TILs	NA	Adult, Senior	Combination Therap	Herlev Hospita	NA	Non-Industry
	19	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT02482090	Jul-15	Apr-17	I	Ovarian Cancer	Metastatic Ova	Solid Tumor	$1x10^{9}$ TILs i	Single dose	Adult, Senior	Combination Therap	Herlev Hospita	NA	Non-Industry
	19	TILs	TIL	NA	Autologous T-Cells	NCT02326053	Dec-16	Dec-18	I	Renal Cell Carcinc	Metastatic Ren	Solid Tumor	NA	Single dose	Adult, Senior	Combination Therap	Herlev Hospita	NA	Non-Industry
New Therapy	20	LTX-315 and TILs	TIL	Intratumoral Injection	Autologous T-Cells	NCT03725605	Dec-18	Feb-23	II	Soft Tissue Sarcor	Advanced/Met	Solid Tumor	NA	NA	Adult, Senior	Combination Therap	Lytix Biopharm	Herlev Hos	Industry / Non-Industry
	21	MILs	TIL	NA	Autologous T-Cells	NCT00566098	Nov-07	Oct-18	III	Multiple Myeloma,	NA	Hematological C	NA	Single dose	Adult, Senior	Combination Therap	Sidney Kimmel	National C	Non-Industry
	22	TILs	TIL	Intravenous Infusion	Autologous T-Cells	NCT01946373	Oct-13	Dec-18	I	Melanoma	Metastatic Mela	Solid Tumor	$5x10^{10}$ TIL	Single dose	Adult, Senior	Combination Therap	Karolinska Uni	NA	Non-Industry
	23	MILs	TIL	NA	Autologous T-Cells	NCT01858558	Sep-13	Jul-24	II	Multiple Myeloma	High Risk Myelc	Hematological C	NA	Single dose	Adult, Senior	Combination Therap	Sidney Kimmel	The Leuke	Non-Industry
	24	TILs	TIL	Intravenous Infusion	NA	NCT00604136	Jul-08	Dec-20	II	Melanoma	Metastatic Mela	Solid Tumor	$1n^{9}$ TIL s	Single dose	Adult, Senior	Combination Therap	Hadassah Mec	NA	Non-Industry

Source: Roots Analysis

Detailed analysis was done on T-cell Immunotherapies. However, it cannot be revealed due to confidentiality purposes.

7

7. T-CELL IMMUNOTHERAPIES: PARTNERSHIPS AND COLLABORATIONS

7.1. CHAPTER OVERVIEW

During our research, we came across 289 instances of partnerships and collaborations that were established between various companies in this domain during the time period 2013-2019. A comprehensive research is carried out to develop database where multiple parameters were captured. The databasedeveloped within this project includes parameters such as:

- Name of the Parent Company
- Parent Company Headquarter
- Name of the Partner Company
- Partner Company Headquarter
- Type of Organization
- Date of Partnership Establishment
- Purpose of Collaboration
- Type of Therapy
- Type of collaboration

7.2. T-CELL IMMUNOTHERAPIES: PARTNERSHIPS AND COLLABORATIONS

Figure 7.1 and 7.2 provide a glimpse of the database of partnerships and collaborations with various parameters captured.

Figure 7.1 **Partnerships and Collaborations Glimpse 1**

S. No.	Parent Company	Parent Company HQ (Country)	Parent Company HQ (Region)	Type of Organization	Partner Company	Partner Company HQ (Country)	Partner Company HQ (Region)	Type of Organization	Month-Year	Therapy / Technology	Product
43	Ziopharm Oncology	US	North America	Industry	TriArm Therapeuti	US	North America	Industry	Dec-18	Sleeping Beauty-gener	NA
44	Ziopharm Oncology	US	North America	Industry	Precigen	US	North America	Industry	Oct-18	Sleeping Beauty (SB) T	NA
45	Merck KGaA	Germany	EU	Industry	Intrexon	US	North America	Industry	Dec-18	Chimeric Antigen Recej	NA
46	Carina Biotech	Australia	Asia Pacific	Industry	Seattle Children's F	US	North America	Non-Industry	Aug-18	Chimeric Antigen Recej	NA
47	MTPConnect	Australia	Asia Pacific	Non-industry	Carina Biotech	Australia	Asia Pacific	Industry	Apr-18	CAR-T immunotherapie	NA
48	Molmed	Italy	EU	Industry	Glycostem	Netherlands	EU	Industry	May-18	NK cells–basedallogen	NA
49	Green Cross Cell	South Korea	Asia Pacific	Industry	Liminatus Pharma	US	North America	Industry	Jul-18	CAR-T immunotherapie	NA
50	MesoBlast	Australia	Asia Pacific	Industry	Cartherics	Australia	Asia Pacific	Industry	May-18	Allogeneic CAR-T	NA
51	Cartherics	Australia	Asia Pacific	Industry	panCELLa	Canada	North America	Industry	Feb-19	FailSafeTM technology	NA
52	TC BioPharm	UK	EU	Industry	NIPRO Corporation	Japan	Asia Pacific	Industry	Feb-18	CAR-based immunothe	NA
53	TC BioPharm	UK	EU	Industry	Trinity College Dul	Ireland	EU	Non-Industry	Feb-19	V delta 1 γδ T cell bank	V delta 1
54	TC BioPharm	UK	EU	Industry	Scotia Biologics	UK	EU	Industry	May-18	novel proprietary tumo	NA
55	Avalon	US	North America	Industry	Arbele	US	North America	Industry	Mar-19	transposon-based Chin	NA
56	Takeda Pharmaceuti	Japan	Asia Pacific	Industry	Memorial Sloan Ke	US	North America	Non-Industry	Jan-19	chimeric antigen recept	NA
57	Takeda Pharmaceuti	Japan	Asia Pacific	Industry	Noile-Immune Biot	Japan	Asia Pacific	Industry	Jan-19	Prime" (proliferation in	NIB-102,
58	Takeda Pharmaceuti	Japan	Asia Pacific	Industry	Crescendo Biologi	UK	EU	Industry	Jan-19	Novel CAR-T therapeu	Humabo
59	Sangamo Therapeut	US	North America	Industry	TxCell	France	EU	Industry	Dec-18	zinc finger nuclease ge	CAR-Tre
60	TxCell	France	EU	Industry	Lonza Pharma & Bi	Switzerland	EU	Industry	Jun-18	CAR-Treg platform	TX200
61	ProMab Biotechnol	US	North America	Industry	ACROBiosystems	US	North America	Industry	Apr-18	CAR-T Therapy	NA
62	Fate Therapeutics	US	North America	Industry	ONO Pharmaceutic	South Korea	Asia Pacific	Industry	Sep-18	pluripotent stem cell (iP	NA
63	Fate Therapeutics	US	North America	Industry	Memorial Sloan Ke	US	North America	Non-Industry	May-18	CRISPR	NA
64	Tessa Therapeutics	Singapore	Asia Pacific	Industry	St. Jude Children's	US	North America	Non-Industry	Sep-18	CAR-expressing Virus-I	NA
65	Tessa Therapeutics	Singapore	Asia Pacific	Industry	MSD	US	North America	Industry	Apr-19	human papillomavirus-I	NA
66	Helix Biopharma	Canada	North America	Industry	Promab Biotechnol	US	North America	Industry	Mar-18	CAR-T Therapy	NA
67	Obsidian Therapeuti	US	North America	Industry	Celgene	US	North America	Industry	Jan-19	Obsidian's Destabilizin	NA

Source: Roots Analysis

Figure 7.2 **Partnerships and CollaborationsGlimpse 2**

S. No.	Parent Company	Partner Company	Partner Company HQ (Country)	Partner Company HQ (Region)	Type of Organization	Month-Year	Therapy / Technology	Product	Type of Therapy	Year	Type of Col	Summary	Link 1	Link 2
43	Ziopharm Oncology	TriArm Therapeuti	US	North America	Industry	Dec-18	Sleeping Beauty-gener	NA	CAR-T	2018	Product De	Ziopharm C	https://ir.ziopharm.com/news-releases/ne	
44	Ziopharm Oncology	Precigen	US	North America	Industry	Oct-18	Sleeping Beauty (SB) T	NA	TCR	2018	Product De	Ziopharm C	https://ir.	https://seekingalpha.com/news/
45	Merck KGaA	Intrexon	US	North America	Industry	Dec-18	Chimeric Antigen Recej	NA	CAR-T	2018	Product De	Intrexon Co	https://in	https://www.merckgroup.com/e
46	Carina Biotech	Seattle Children's F	US	North America	Non-Industry	Aug-18	Chimeric Antigen Recej	NA	CAR-T	2018	Clinical Tri	arina Biotec	http://carinabiotech.com/car-t-trial-for-soli	
47	MTPConnect	Carina Biotech	Australia	Asia Pacific	Industry	Apr-18	CAR-T immunotherapie	NA	CAR-T	2018	Product De	This fantas	http://carinabiotech.com/carina-receives-n	
48	Molmed	Glycostem	Netherlands	EU	Industry	May-18	NK cells–basedallogen	NA	CAR-T	2018	Manufactu	MolMed S	http://glycostem.com/ cache/glycostem/m	
49	Green Cross Cell	Liminatus Pharma	US	North America	Industry	Jul-18	CAR-T immunotherapie	NA	CAR-T	2018	Other	GC Cell, a S	http://m.ajudaily.com/view/2018072415580	
50	MesoBlast	Cartherics	Australia	Asia Pacific	Industry	May-18	Allogeneic CAR-T	NA	CAR-T	2018	Product De	Mesoblast	https://www.globenewswire.com/news-re	
51	Cartherics	panCELLa	Canada	North America	Industry	Feb-19	FailSafeTM technology	NA	CAR-T	2019	Product De	Cartherics F	https://markets.businessinsider.com/news	
52	TC BioPharm	NIPRO Corporation	Japan	Asia Pacific	Industry	Feb-18	CAR-based immunothe	NA	CAR-T	2018	Product De	The NIPRO	http://ww	https://ncltv.co.uk/news/scotlan
53	TC BioPharm	Trinity College Dul	Ireland	EU	Non-Industry	Feb-19	V delta 1 γδ T cell bank	V delta 1	CAR-T	2019	Product De	TC BioPhar	http://www.tcbiopharm.com/index.php/co	
54	TC BioPharm	Scotia Biologics	UK	EU	Industry	May-18	novel proprietary tumo	NA	CAR-T	2018	Product De	TC BioPhar	http://www.tcbiopharm.com/index.php/co	
55	Avalon	Arbele	US	North America	Industry	Mar-19	transposon-based Chin	NA	CAR-T	2019	Other	Avalon Glo	https://www.contractpharma.com/content	
56	Takeda Pharmaceuti	Memorial Sloan Ke	US	North America	Non-Industry	Jan-19	chimeric antigen recept	NA	CAR-T	2019	Product De	Takeda will	https://w	https://www.cancertherapyadvis
57	Takeda Pharmaceuti	Noile-Immune Biot	Japan	Asia Pacific	Industry	Jan-19	Prime" (proliferation in	NIB-102,	CAR-T	2019	Product Lic	Takeda exe	https://www.takeda.com/newsroom/news	
58	Takeda Pharmaceuti	Crescendo Biologi	UK	EU	Industry	Jan-19	Novel CAR-T therapeu	Humabo	CAR-T	2019	Product Lic	Takeda's ex	https://www.takeda.com/newsroom/news	
59	Sangamo Therapeut	TxCell	France	EU	Industry	Dec-18	zinc finger nuclease ge	CAR-Tre	CAR-T	2018	Acquisition	The acquisi	https://in	https://investor.sangamo.com/n
60	TxCell	Lonza Pharma & Bi	Switzerland	EU	Industry	Jun-18	CAR-Treg platform	TX200	CAR-T	2018	Manufactu	TxCell and I	https://www.epmmagazine.com/news/txce	
61	ProMab Biotechnol	ACROBiosystems	US	North America	Industry	Apr-18	CAR-T Therapy	NA	CAR-T	2018	R&D Agree	ACROBios	https://www.acrobiosystems.com/A1082-A	
62	Fate Therapeutics	ONO Pharmaceutic	South Korea	Asia Pacific	Industry	Sep-18	pluripotent stem cell (iP	NA	CAR-T	2018	Product De	Fate Therap	https://ir.fatetherapeutics.com/news-relea	
63	Fate Therapeutics	Memorial Sloan Ke	US	North America	Non-Industry	May-18	CRISPR	NA	CAR-T	2018	Other	Fate Therap	https://ir.fatetherapeutics.com/news-relea	
64	Tessa Therapeutics	St. Jude Children's	US	North America	Non-Industry	Sep-18	CAR-expressing Virus-I	NA	CAR-T	2018	Clinical Tri	St. Jude Ch	https://www.prnewswire.com/news-releas	
65	Tessa Therapeutics	MSD	US	North America	Industry	Apr-19	human papillomavirus-I	NA	CAR-T, TCR, TIL	2019	Clinical Tri	Tessa Ther	https://www.tessatherapeutics.com/2019/(	
66	Helix Biopharma	Promab Biotechnol	US	North America	Industry	Mar-18	CAR-T Therapy	NA	CAR-T	2018	Product De	Helix BioPh	https://www.globenewswire.com/news-re	
67	Obsidian Therapeuti	Celgene	US	North America	Industry	Jan-19	Obsidian's Destabilizin	NA	CAR-T	2019	R&D Agree	Obsidian Th	https://ob	https://www.businesswire.com/

Source: Roots Analysis

Detailed analysis on partnership and collaboration instances have been included in the project report. However, it cannot be revealed due to confidentiality purposes.

8

8. T-CELLIMMUNOTHERAPIES: COST PRICE ANALYSIS

8.1. CHAPTER OVERVIEW

During our research, we came across two therapies that have been commercialized in various regions across the globe.Further, continuous efforts are being made to prove the therapeutic potential of several other candidate therapies and to overcome the existing challenges associated with manufacturing and pricing. Several pharmaceutical companies have collaborated with academia to develop / fund the development of these therapies.A comprehensive research is carried out to highlight on the various factors that must be taken into consideration while deciding the prices of cell-based therapies. The analysis features discussions on different models / approaches that a pharmaceutical company may choose to follow to decide the price at which their T-cell based immunotherapy product can be marketed.

8.2. T-CELL IMMUNOTHERAPIES: FACTORS CONTRIBUTING TOWARDS HIGH PRICE OF THERAPIES

The gene therapies are known to be priced much higher as compared to other conventional drugs.For the price analysis of T-cell immunotherapies, we identified a few cell therapy products, along with their treatment costs.The therapies considered for the analysis were similar to T-cellimmunotherapies, in terms of manufacturing and treatment procedures. Figure 8.1 provides a glimpse of the list of therapies with cost of therapy per treatment.

Figure 8.1 **Cost Price Analysis Glimpse 1**

S. No	Product Name	Company Sponsor	Type of Therapy	Indication	Treatment Cost
1	Cartistem[20]	Medpost	Stem Cell Therapy	Osteoarthritis	USD 20,000-40,000[21]
2	ChondroCelect[22]	TiGenix	Cell Therapy	Cartilage regeneration	USD 24,000[23]
3	Cupistem[24]	Anterogen	Stem Cell Therapy	Rectal fistula	USD 3,000-5,000[25]
4	Gendicine[26]	Shenzhen SiBiono GeneTech	Gene Therapy	Solid tumors	USD 100,000[27]

Source: Roots Analysis

The oncology market remains very active and is moving towards targeted treatments and combination therapies. Therapies are developed for indications, for which drugs are been approved. Figure 8.2 provides a list of some of the targeted drugs along with their cost

Figure 8.2 **Cost Price AnalysisGlimpse 2**

68	Xermelo[207] (telotristat ethyl)	Lexicon | Pharmaceuticals	Tryptophan hydroxylase protein inhibitor	Carcinoid syndrome diarrhea	USD 61,000 - 72,000 / year[208]
69	Xospata[209] (gilteritinib)	Astellas	Kinase Inhibitor	Acute myeloid leukemia	USD 22,500 / month[210]
70	Xtandi[211] (enzalutamise)	Astellas	Androgen Receptor Inhibitor	Castration-resistant prostate cancer	USD 60,000 / year[212]
71	Yervoy (ipilimumab)	BMS	Monoclonal Antibody	Melanoma	USD 120,000 / treatment[213],[214]
72	Zelboraf[215] (vemurafenib)	Roche	BRAF enzyme Inhibitor	Erdheim-chester disease	USD 136,000 / year[216]

Source: Roots Analysis

The high prices of treatment options for such indications continues to be one of the key challenges associated with the adoption of these therapies by the patients. An analysis based on the size of patient population was done using the pricing model. Further estimated prices for T-cell immunotherapies, as predicted / estimated by other analyst / experts within the immune-oncology field was listed to highlight the views of experts in this domain.

Details on other factors contributing towards high price of T-cell therapieshave been included in the project report.However, it cannot be revealed due to confidentiality purposes.

9

9. T-CELL IMMUNOTHERAPIES: PROMOTIONAL ANALYSIS

9.1. CHAPTER OVERVIEW

Drug developers heavily rely on a number of broadcasting channels, such as direct to consumer (DTC) advertisements, product websites and conferences, to promote the use of their drugs. The importance of such promotions is evident from the fact that the pharmaceutical industry spent close to USD 6.1 billion in DTC advertisements in 2017.[107] The target individuals for these promotional campaigns are the consumers (patients), caretakers / caregivers and healthcare professionals (physicians). However, it is important to highlight thatDTC advertising of prescription drugs is permitted only in the US, New Zealand, Bangladesh and South Korea. In other geographies, such as Europe, these promotional campaigns are not allowed as it is believed that advertisements may put physicians in a situation where they have to prescribe the advertised drug based on patients' demands (even if they are not the better alternatives).[108, 109]Moreover, the increase in healthcare costs related to promotional activities is another concern, as the medications are completely reimbursed by the government in some countries.

Promotional campaigns offer a number of advantages to drug developers, end users and physicians,some of which are listed below:[110]

- They play an important role in the adoption of the product
- They serve as one of the factors that affect medical practices (by influencing physicians) and the interaction of patients with physicians
- They inform, educate and empower the patients
- These campaigns encourage patients to contact a clinician
- They drive patient compliance
- They strengthenrelationship between patients and clinicians

[107] Source: https://www.mmm-online.com/commercial/dtc-pharma-ad-spending-slipped-46-in-2017-kantar/article/750421/
[108] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2661977/
[109]Source: http://www.pharmtech.com/will-dtc-advertising-appear-europe?id=&pageID=1&sk=&date
[110]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278148/

- They help in reduction of underdiagnoses and undertreatment of indications
- They help in curbing drug prices by encouraging product competition

This chapter elaborates on the key promotional strategies being adopted by the developers of the recently approved CAR-T therapies, Kymriah for the treatment of r/r ALL and r/r DLBCL and Yescarta for the treatment of r/r NHL. The promotional aspects covered in the chapter include a product website analysis (covering key messages for patients and healthcare professionals), patient assistance programs and the presence of the developers in various conferences.It also provides a comparative analysis of promotional activities for the two drugs (mentioned above). In addition, the chapter includes a brief overview of the different channels used for these promotional campaigns.

9.2. Channels Used for Promotional Campaigns

Figure 9.1 provides a snapshot of various channels that are available for use by drug developers to promote their products.

Figure9.1 Channels Used for Promotional Campaigns

Source:Roots Analysis

Some of these channels have been outlined below:

- **Product websites:** In order to provide the necessary drug-related information to patients and healthcare professionals, drug developers launch product specific websites. The primary objective of such websites is to provide details on different aspects of the drug,

such as indications for which the product has been approved, its efficacy benefits and the safety concerns.[111]

- **Patient assistance programs:**[112]Drug developersgenerally initiate patient assistance programs in order to help the under privileged patient groups to access their drugs. The drug developers offer co-pay schemes or provide the drug free-of-cost for a specified period of time. The primary objective of such patient assistance programs is to support patients financially, as well as to provide them with living assistance during the course of the treatment.[113]
- **Detailing material (face to face sales and promotional activities):** Detailing material, such as leaflets and brochures, containing information on the drug, is another promotional strategy that is used by pharmaceutical companies. The objective of this strategy is to educate physicians about the product, with the expectation that the physician will prescribe the drug to his / her patients suffering from the indication for which the drug is approved.[114]
- **DTC advertisements:** Promotional campaigns of prescription products that directly target the consumers (patients) are known as DTC advertisements.Drug developers make use of popular media platforms, such as television, print media (magazines and newspapers) and the radio to advertise their products. The main objective is to make patients familiar with the product and provide information on indication(s) for which it can be used, along with efficacy and / or safety results.[115]
- **Oral / poster presentations at conferences:**Conferences that are held at the national / international level provide opportunities to drug developers to present their clinical findings generated from different clinical studies of the drug. Companies participate in such conferences to spread awareness about their drug among healthcare professionals.
- **Marketing through influencers:** Any person having huge followers on social media platform is called an influencer. Pharmaceutical companies can employ these influencers to promote their products to a large fan base of the influencers.[116]
- **Messaging applications:** Pharmaceutical industries can promote their products by using various messaging applications to influence the target customers with valuable and reliable content. These applications offer an opportunity to drug developers to have a one-to-one discussion with doctors and patients.[117]

[111]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278148/
[112] Patient assistance programs are primarily available for US residents only
[113]Source: https://www.needymeds.org/article
[114]Source: https://searchhealthit.techtarget.com/definition/detailing
[115]Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3278148/
[116]Source: https://www.businesswire.com/news/home/20180727005267/en/Types-Marketing-Strategies-Pharmaceutical-Companies-Boost-Profits
[117]Source: https://www.businesswire.com/news/home/20180727005267/en/Types-Marketing-Strategies-Pharmaceutical-Companies-Boost-Profits

- **Chatbots:** These are automated preprogrammed scripts that imitate human behavior, and are used for direct communication with the customers. It is one of the most compelling strategies used by pharmaceutical industries to reply and involve with clients. It helps in handling frequently asked questions (FAQs), questionnaires, and surveys. Additionally, chatbots are utilized to offer personalized services and to automate several time-consuming processes.[118]

Detailed analysisof product websites of two approved therapies, Kymriah and Yescarta, have been included in the project report. However, it cannot be revealed due to confidentiality purposes.

[118]Source: https://www.businesswire.com/news/home/20180727005267/en/Types-Marketing-Strategies-Pharmaceutical-Companies-Boost-Profits

10

10. T-CELL IMMUNOTHERAPIES: EMERGING TECHNOLOGIES

10.1. CHAPTER OVERVIEW

Over the years, the increasing popularity of immunotherapies has paved the way for the discovery and development of several novel technology platforms. Most of these innovative technologies have the potential to be applied in the production of superior CAR-Ts, advanced TCR constructs and other such targeted therapeutic systems. Technologies for genome sequencing, genome editing and other molecular cell manipulation systems, such as switches and transposons, have significantly impacted the T-cell therapy domain.

This chapter provides details of the emerging technologies and platforms that have helped further research in the field of T-cell immunotherapies. It includes comprehensive descriptions of the various technologies, key collaborations related to each of them, their various applications, and advantages / disadvantages. Since most of the technologies described in this chapter are owned by start-ups and small firms, we have also provided information about the various venture capital investments that have driven such initiatives in the past few years. It is worth mentioning that the funding instances mentioned in the chapter are specific to the company and not to the T-cell therapy domain.

10.2. GENOME EDITING TECHNOLOGIES

10.2.1. TECHNOLOGY OVERVIEW

Gene editing refers to the process of modifying a single gene or a set of genes within the genome of an organism by altering the nucleotide sequence using specialized molecular tools, such as artificially engineered nucleases or molecular scissors.[119] Basically, there are three ways in which genes can be manipulated, namely:[120]

- Gene Insertion: This involves the addition of new attributes to a gene through the incorporation of nucleotide sequences.

[119]Source:https://www.horizondiscovery.com/gene-editing
[120]Source: https://www.yourgenome.org/facts/what-is-genome-editing

- Gene Repair: This refers to the replacement of a defective gene sequence by a functional sequence.
- Gene Inactivation: This involves the use of specific nucleotide sequences or regulatory elements to prevent the expression of a target gene.

It is worth mentioning that various non-profit organizations, such as the Innovative Genome Institute (IGI), were launched with an aim to accelerate the adoption of innovative technologies that have surfaced as a viable growth driver for genome editing. Particularly, the IGI came into being in 2014 as Innovative Genomics Initiativewhich was formed by partnerships of various universities, including the University of California, Berkeley and the University of California, San Francisco. It focused on unfolding the mechanism of actions masking CRISPR-based genome editing and applying this technology to improve human health and their welfare. In 2015 few philanthropic donations helped in broadening theIGI vision and mission. In January 2017, IGI was officially re-launched as Innovative Genomics Institute.IGI is dedicated to supporting research in genome editing across academia and the biopharmaceutical industry.[121]

We have come across various companies that have developed their own gene editing technology platforms for developing gene therapy. Some of them have even licensed their technology to other companies for developing gene therapies.

10.2.2. APPLICATIONS

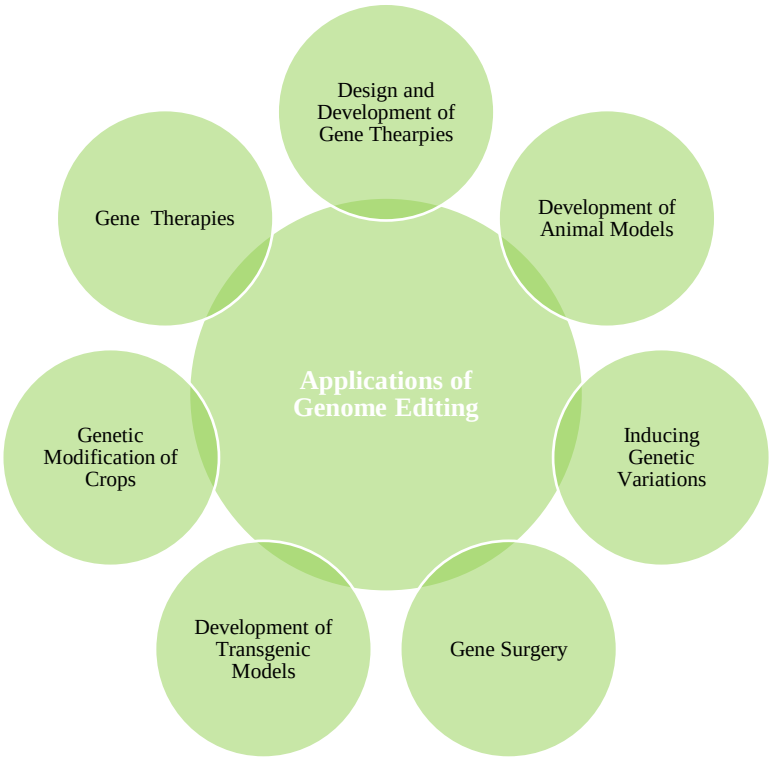
The applications of gene editing in modern medicine are numerous, some are listed below[122]:

- Manipulation of genes for research purposes[123]
- Rapid generation of transgenic models[124]
- Generation of cellular models for tracking the cause / etiology of major diseases, such as diabetes, heart diseases, schizophrenia and autism[125]
- Identification and characterization of novel genes from a functional genome using unbiased screening[126]
- Production of advanced therapies (such as gene therapies)[127]

Figure 10.1 is a pictorial representation of the various applications of genome editing.

[121]Source: https://innovativegenomics.org/overview/
[122] Source: http://www.ddw-online.com/enabling-technologies/p149526-editing-the-human-genome:-role-in-functional-genomics-and-translational-medicine-summer-12.html
[123] Source: http://www.sciencedirect.com/science/article/pii/S1525001616309613
[124] Source: http://ko.cwru.edu/publications/hsucrisprreview.pdf
[125] Source: http://ko.cwru.edu/publications/hsucrisprreview.pdf
[126] Source: http://ko.cwru.edu/publications/hsucrisprreview.pdf
[127] Source:https://www.ncbi.nlm.nih.gov/pubmed/25398345

Figure 10.1 Genome Editing Technologies: Applications

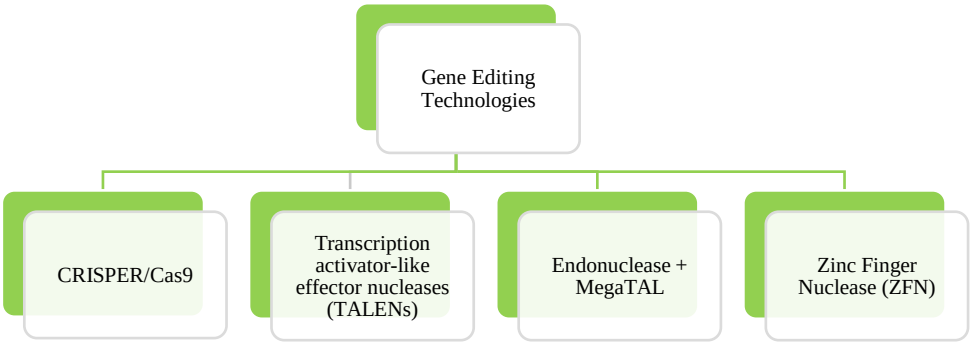


Source: Roots Analysis

10.3. EMERGING TECHNOLOGY PLATFORMS USED IN T-CELL THERAPIES

Figure 10.2 lists some of the major gene editing platforms that are being used for the development of various T-cell therapies.

Figure 10.2 Genome Editing: Emerging Technology Platforms Used in T-CellTherapies



Source: Roots Analysis

10.3.1. CRISPR/CAS9 SYSTEM

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) genes were first described in 1987. They were initially discovered in *Escherichia coli* and were observed to be short repeats of DNA within the primitive bacterial genome. However, at that stage researchers were highly uncertain of the exact function of such sequences. Later, studies revealed that these sequences enabled the organism to respond to and eliminate invading genetic material. The bacteria make use of these sequences to tag viral DNA, which was incorporated in their genomes, using the Cas system. Once tagged with CRISPR sequences, the viral genome could be easily traced within the host genome, and selectively eliminated. In other words, the CRISPR / Cas9 system formed a primitive defense mechanism against viral attack, and the bacteria possessing such molecular tools were rendered resistant to viral infections. The exact mechanism of action of the CRISPR / Cas9 system was elucidated in 2007 by Rodolphe Barrangou[128]and his colleagues Through an experiment conducted in *Streptococcus thermophilus*, they demonstrated that bacteria were capable of acquiring resistance to viral infection when the DNA of an infectious phage was integrated within the CRISPR locus of the bacterial genome.[129, 130]

The CRISPR / Cas9 system has revolutionized the field of genetic engineering. It enables researchers to alter the genomes of a range of organisms with relative ease.[131] Currently, it has emerged as a promising tool that is used extensively for editing mammalian genomes, and for the development of novel treatment options. It is worth mentioning that the technology has significantly improved over the years.[132]

In April 2014, the USPTO approved the first patent related to this technology, which was filed by the Broad Institute and Massachusetts Institute of Technology.[133] The patent (US 8,697,359) protects the CRISPR / Cas9 system and also covers the methods for using the system for gene manipulation.

10.3.1.1. KEY COMPONENTS AND FUNCTION

The CRISPR/Cas9 system comprises of the following components, which are all crucial to the structural integrity and function of the entire system:[134]

[128], Rodolphe Barrangou became first author to publish a paper providing experimental proof for the immune function of CRISPR in *Science*, in 2007. He has also worked on Cas9 guided RNA characterization and has been awarded 17 patents as of 2016. Currently, he is Editor-in-Chief of The *CRISPR* Journal, which debuted in February 2018 and working in the North Carolina State University as an Associate Professor
[129] Source: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
[130] Source: http://labiotech.eu/review-crispr-therapeutical-revolution/
[131] Source: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
[132]Source: https://labiotech.eu/review-crispr-therapeutical-revolution/
[133] Source: http://editasmedicine.com/documents/Broad%20Institute%20awarded%20first%20patent%20for%20engineered%20CRISPR.pdf
[134]Source:http://ko.cwru.edu/publications/hsucrisprreview.pdf

- A short guide RNA sequence, which is a partly conserved palindromic sequence that directs Cas9 to a specific DNA sequence in the genome and convenes double strand breakage within the target gene
- A DNA specific CRISPR RNA (crRNA) sequence
- The Cas9 protein, which is responsible for catalyzingene replacement through homologous recombination

In certain cases, an additional trans-activating CRISPR RNA (tracrRNA) is required to facilitate the Cas9-DNA-RNaseIII interaction. Cas9 acts as the host to the RNA sequence and helps position it on the target DNA and thereafter, nicks both the strands at positions corresponding to the guide sequence.[135]It is worth highlighting that the CRISPR-Cas9 complex can be directed towards different genes as required. This is done by exploiting the CRISPR mechanism of action and modifying the Cas9 protein to either specifically activate or repress the expression of a target gene.[136]

10.3.1.2. MECHANISM OF ACTION

The key steps involved in the CRISPR/Cas mediated defense mechanism within bacteria are mentioned below:[137]

- **Adaption:**Insertion of new gene sequences (primarily, attacking viral genomes) within the CRISPR locus in the bacterial genome.
- **Expression:** Transcription of the CRISPR locus, followed by processing of the newly synthesized crRNA.
- **Interference:** crRNA mediated detection of target gene sequence and selective elimination by Cas protein(s).

Based on the way crRNA is processed and the method of interference involved, three types of CRISPR/Cas mechanisms have been identified.[138]

- **Type I Systems:** In these systems, Cas5 or Cas6 mediates the processing of pre-crRNA and Cas3 is involved in the interference step along with the Cascade complex and the mature crRNA.
- **Type II Systems:** In these systems, tracrRNA, RNaseIII and an unknown factor are responsible for pre-crRNA processing. More specifically, the complex mediates 5' end trimming. In this case, Cas9, along with crRNA facilitates the interference process. It is

[135] Source: https://labiotech.eu/review-crispr-therapeutical-revolution/
[136]Source: https://labiotech.eu/review-crispr-therapeutical-revolution/
[137]Source: http://www.sciencedirect.com/science/article/pii/S0300908415001042
[138]Source: http://www.sciencedirect.com/science/article/pii/S0300908415001042

worth mentioning that this is the most extensively researched and well understood mechanism when it comes to the CRISPR/Cas mechanism.

- **Type III Systems:** Similar to type I systems, these systems also use Cas6 for processing pre-crRNA. However, in this case, an unknown factor mediates 3' end trimming of the pre-crRNA. In type III systems, the type III Csm/Csr complex is involved in the DNA interference process. Additionally, researchers believe that this is capable of targeting RNA as well.

10.3.1.3. TARGETING EFFICIENCY AND CHALLENGES

Targeting efficiency of gene editing tool refers to the percentage of desired mutations achieved by the same. It is considered as one of the key parameters for assessing a genome manipulation tool. Studies have shown that Cas9 demonstrates a similar efficiency as displayed by other gene editing tools, such as TALENs and zinc finger nuclease (ZFNs). In humans, TALENs and ZFNs show efficacies ranging from 1-50%, which is much more than that of Cas9. Although the Cas9 system demonstrates high targeting efficiency in zebra fish and plants (>70%), its efficiency is as low as only 2-5% in induced pluripotent stem cells. Studies have demonstrated that the use of dual single-guide RNA (sgRNA) sequences can improve genome targeting by up to 78% in single-cell mouse embryos.[139]

One of the other challenges associated with the use of this technology includes the risk of off-target mutations. Conventionally, lesser the occurrence of off-target mutations, more efficient is a gene editing system. Currently, two methods have been devised to reduce the occurrence of such unwanted mutations. One method involves the use of a truncated guide RNA sequence (within the crRNA-derived sequence) or the addition of two extra guanine (G) nucleotides to the 5´end of the crRNA. The second method makes use of paired nickases. The strategy utilizes D10A Cas9 and two sgRNAs complementary to the adjacent area on opposite strands of the target site for reducing off-target mutations.[140]

10.3.1.4. NEXT-GEN CRISPR TECHNOLOGY

The Next-GEN CRISPR technology is a first-in-class gene editing tool that features a truly dimeric RNA-guided nuclease. The technology was developed by Transposagen Biopharmaceuticals.[141] In addition to possessing all the benefits of the CRISPR/Cas9 editing

[139]Source: https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
[140]Source:https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology
[141]Source: Company's Website

tool, the technology has several added advantages. Some of the salient features of this technology, as claimed by the developers, are listed below.[142]

- Simplicity
- Precision and fidelity
- Ease of design and use
- High efficiency
- Multiplexibility
- Elimination of off-site mutation problems
- Increase in the number of targeting sites

Details on other emerging technologies related to T-cell therapies have been included in the project report. However, it cannot be revealed due to confidentiality purposes.

[142]Source:http://www.transposagenbio.com/news-events/transposagens-nextgen-crispr-technology-promises-clean-genome-editing-without-off-target-mutations

11

11. FUTURE SCOPE OF THE WORK

In addition to the above-mentioned chapters, the future workplan of the project is structured as below:

- An analysis of the CAR constructs of clinical CAR-T therapies based on generation of CAR-T therapy (first generation, second generation, third generation and fourth generation), type of binding domain (murine, humanized, fully human and rabbit derived), type of vector and type of co-stimulatory domain used.
- An analysis of the global CAR-T clinical trials registered between 2009 and 2019, highlighting the year wise trend and the distribution across different geographies.
- An overview of the various focus therapeutic areas of therapy developers, including an assessment of the opportunity offered by oncological and non-oncological disease indications.
- An analysis of the investments that have been made into companies that have proprietary products / technologies, including seed financing, venture capital financing, capital raised from IPOs and subsequent offerings, grants and debt financing.
- A case study on other T-cell based therapies, apart from CAR-Ts, TCRs and TILs. It presents a detailed analysis of the approved / pipeline products in this domain, including information on the current phase of development, target therapeutic areas, type of T-cells used, and source of T-cells.
- A case study on manufacturing cell therapy products, highlighting the key challenges, and a list of contract service providers and in-house manufacturers that are involved in this space.
- To estimate the potential sales of T-cell immunotherapies that are currently marketed or are in late stages of development. Additionally, the chapter presents a detailed market segmentation on the basis of type of therapy (CAR-T, TCR and TIL), geography (North America, Europe and Asia Pacific) and target indications (acute lymphoblastic leukemia, acute myeloid leukemia, bladder cancer, cervical carcinoma, chronic lymphocytic leukemia, esophageal cancer, head and neck cancer, multiple myeloma, hepatocellular carcinoma, melanoma, non-Hodgkin’s lymphoma, non-small cell lung cancer, ovarian cancer and synovial sarcoma).

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12. CONCLUSION

Immunotherapies arethe fourth major pillar of cancer therapy, after surgery, chemotherapy and radiotherapy. Based on the principle of utilizing the body's natural defense system comprising of immune cells (such as T-cells and B-cells) and proteinaceous mediators (such as cytokines and complement system proteins) to combat diseases, these therapieshave emerged as a potent and viable therapeutic intervention. Presently, the T-cell immunotherapies market is characterized by the presence of two approved therapies and over 600candidates in clinical / preclinical stages of development. Encouraging clinical results and therapeutic response rates achieved across various hematological cancers and solid tumors have inspired research groups to presently focus their efforts in these therapeutic areas.

At present, more than 80% of pipeline T-cell therapies are being developed to target thetherapeutic areas. Particularly for CAR-T therapies, the initial focus was mainly on hematological malignancies, such as ALL, NHL, MM, CLL and AML. However, of late, several CAR-T therapies are being developed for the treatment of solid tumors, such as pancreatic cancer, glioblastoma, hepatocellular carcinoma, breast cancer, lung cancer, neuroblastoma ovarian cancer, colorectal cancer and ovarian cancer, as well. In contrast, TCRs and TILs have been shown to be particularly effective against solid tumors, such as melanoma, lung cancer, ovarian cancer, sarcoma, bladder cancer, esophageal cancer, breast cancer, and head and neck cancer.In addition to oncological indications, active R&D efforts are underway to develop T-cell based therapies for infectious diseases and autoimmune disorders as well.

With an aim to develop T-cell immunotherapy products with improved efficacy and safety profiles, various technology providers have developed innovative and advanced platforms. These include technologies for genome sequencing, genome editing and other molecular-level cell manipulation systems, such as switches and transposons. These scientific advancements are considered as significant value additions to the field and are expected to advance the discovery and development of T-cell immunotherapies.

T-cell immunotherapy offers hope tothe patients suffering from late stage cancers, which cannot be efficiently treated with existing treatment modalities. Some of the key drivers of the market include the increase in the number of collaborations being inked between non-industry and industry players, emergence of innovative technology platforms, lucrative rounds of VC funding and encouraging clinical results. Innovation-driven research programs, discovery of several novel targets and a growing pipeline are also anticipated to contribute to the further growth of this market.Withseveral promising candidates in the development pipeline targeting major therapeutic areas, the market is poised for success in the long-run as multiple product candidates are expected to get approved over the coming decade. In general, there is a broad industry consensus that these therapies, when approved, are likely to achieve blockbuster status in a very short span of time.