“Competitive Insights
on the
Oral Proteins and Peptides Market
and
Viral Vector and Plasmid DNA Manufacturing Market”

Dissertation submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY
IN
BIOTECHNOLOGY

By
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Under the guidance of

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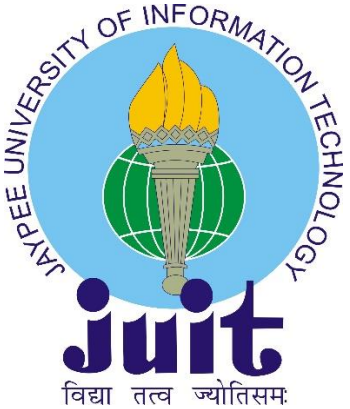

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY, WAKNAGHAT

May 2018

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DECLARATION BY THE SCHOLAR

I hereby declare that the work reported in the B-Tech thesis entitled **"Competitive Insights** on the **Oral Proteins and Peptides Market** and **Viral Vector and Plasmid DNA Manufacturing Market"** submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the supervision of **Snehasish Das**. I have not submitted this work elsewhere for any other degree or diploma.

(Nayanika Chakravarty)

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Date:

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(Snehasish Das)

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SUPERVISOR CERTIFICATE

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Nayanika Chakravarty

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LIST OF ACRONYMS AND ABBREVIATIONS

1.	AAV	Adeno Associated Virus
2.	CMO	Contract Manufacturing Organization
3.	CMV	Cytomegalovirus
4.	GAM	Gene Activated Matrix
5.	GI	Gastrointestinal
6.	GMP	Good Manufacturing Services
7.	IBS-C	Irritable Bowel Syndrome with Constipation
8.	IPO	Initial Public Offering
9.	ITR	Inverted Terminal Repeats
10.	NIH	National Institutes of Health
11.	ORF	Open Reading Frame
12.	R&D	Research and Development
13.	RME	Receptor Mediated Endocytosis
14.	SEC	Securities and Exchange Commission

CHAPTER 1

INTRODUCTION

1.1. ABOUT ROOTS ANALYSIS

Roots Analysis Pvt. Ltd. specializes in providing in-depth business research and consulting services for the pharmaceutical industry. The main focus of the company is on forecasting the market opportunities and future trends for specialized industry players. Apart from this, the reports made by Roots Analysis provide independent views on various aspects of a project, such as the evolving technologies, research & development, future commercial potential, regulatory concerns and risks and opportunities that are associated with the same.

1.2. TRAINING HIGHLIGHTS

1.2.1. RESEARCH METHODOLOGY

The data presented in this report has been gathered via secondary and primary research. For all our projects, we conduct 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 evolve 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:

- Annual reports
- Investor presentations
- SEC filings
- Industry databases
- News releases from company websites
- Government policy documents
- Industry analysts' views

While the focus has been on forecasting the market till 2030, the report also provides our independent view on various trends 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.

1.3. TOPIC AND SCOPE OF THE THESIS

Over the course of my training at Roots Analysis Pvt. Ltd., which started on 2nd February 2018, I was entitled to work on two projects.

- Oral Proteins and Peptides Market: Funding and Investment Analysis
- Vector Manufacturing Market: Partnerships and Collaborations

1.3.1. ORAL PROTEINS AND PEPTIDES MARKET: FUNDING AND INVESTMENT ANALYSIS

Therapeutics based on proteins and peptides have been in use for several decades since the approval of the first protein therapy, recombinant human insulin in 1982. Earlier, subcutaneous injection was the most commonly used route for the delivery of biologic drugs. However, with advances in delivery formulations, over time, have enabled the development of orally administrable versions of therapeutic proteins / peptides.

This project focuses on the growing popularity of oral proteins and peptides based therapeutics. It includes a detailed analysis of the investments made at various stages of development in companies that are focused in this area, including seed financing, venture capital financing, debt financing, grants, capital raised from IPOs and subsequent offerings. Such analyses are important because they help evaluate past investment decisions, predict future returns, assess the scope of a product and the likely performance of a company.

1.3.2. VECTOR MANUFACTURING: PARTNERSHIPS AND COLLABORATIONS, 2018-2030

Today, genetically modified therapies are considered a promising treatment option for various chronic indications, such as Alzheimer's disease, Parkinson's disease and rheumatoid arthritis, among others. In these therapies that involve genetic modification, a therapeutic DNA / gene of interest is introduced into a patient's body / cells. This process is accomplished by the use of vectors. Over time, various viral and non-viral vectors have been developed, optimized and standardized for the same. Presently, the most popular viral vectors are based on AAV, adenovirus, lentivirus and retrovirus, on the basis of their use in active clinical trials. On the other hand, amongst non-viral vectors, plasmid DNA has emerged as the most suitable option. Plasmid DNA is also used in the development and production of viral vectors and DNA vaccines.

This project offers a comprehensive study of the current scenario of manufacturing of viral and non-viral vectors that are primarily used for the development of gene therapies and T-cell therapies. It features an in-depth analysis of the recent collaborations (since 2015) that are based on vector manufacturing on the basis of year in which the agreement was signed, type of agreement, type of vector, and scale of operation (laboratory, clinical and commercial).

ORAL PROTEINS AND PEPTIDES MARKET: FUNDING AND INVESTMENT ANALYSIS

CHAPTER 2

INTRODUCTION TO ORAL PROTEINS AND PEPTIDES

2.1. CHAPTER OVERVIEW

This chapter presents a detailed comparison of the key characteristics of small molecules and biologics. Further, it includes a discussion on proteins and peptides, highlighting their potential as therapeutic agents. This is followed by a brief discussion on the various routes of administration used for drug delivery, with an emphasis on the oral delivery of proteins / peptides. Further, it elaborates on the advantages and challenges associated with the development and delivery of orally administrable formulations of biologics. In addition, the chapter features a comprehensive discussion on the various approaches used for the effective delivery of oral proteins / peptides.

2.2. INTRODUCTION TO PROTEINS

Proteins are large biomolecules that have been shown to play a pivotal role in regulatory, structural and functional mechanisms within cells, tissues and organs in the body. These are considered to be the most abundant organic molecules in biological systems. Proteins consist of sequence of α-amino acids, which are joined through peptide linkages.[1] These amino acids are primarily made up of carbon, oxygen, nitrogen and sulfur.[2]

2.2.1. PROTEIN STRUCTURE

Proteins are highly complex structural entities that are present in all living organisms and found in all compartments of living cells. They constitute more than 50% of an organism's dry weight.[3]Proteins vary in structure even when present in same cell type, and thereby, are capable of performing different functions. The different levels of the structural organization of a protein have been discussed below:[4]

Primary Structure: The primary structure of a protein refers to the sequence of amino acids that are covalently linked to form the polypeptide chain. This sequence, which is encoded in the corresponding gene, determines the structure and function of the protein.

Secondary Structure: The spatial conformation of amino acids is called the secondary structure of a protein. It is determined by the pattern of hydrogen bonding between the amino nitrogen and

[1] Source: https://ghr.nlm.nih.gov/primer/howgeneswork/protein
[2] Source: http://www.ijpsonline.com/articles/properties-and-formulation-of-oral-drug-delivery-systems-of-protein-and-peptides.html
[3] Source: http://www.tuscany-diet.net/proteins/definition-composition-structure/
[4] Source: http://www.tuscany-diet.net/proteins/definition-composition-structure/

carboxyl oxygen of amino acids in a polypeptide chain. Depending on this hydrogen bonding pattern, there are primarily two types of secondary structures, namely α-helices and β-pleated sheets.

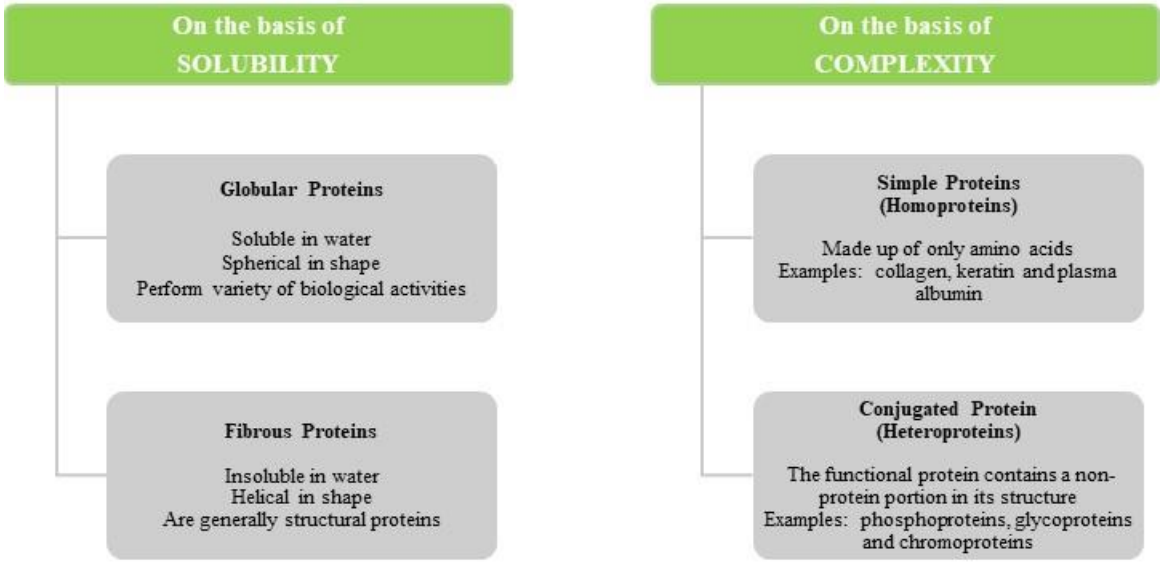
Tertiary Structure: The native three-dimensional configuration of a protein is called its tertiary structure. This structure is stabilized by various interactions between the side chains of amino acids; these include hydrophobic interactions, hydrophilic interactions, hydrogen bonds and di-sulphide bridges between cysteine residues.

Quaternary Structure: The association of two or more polypeptides constitutes the quaternary structure of a protein molecule. Such complexes are typically held together by non-covalent bonds.[5]

2.2.2. CLASSIFICATION

Figure 2.1 highlights the various classes of proteins based on their solubility and complexity.

Figure 2.1 **Classification of Proteins**



Source: https://www.future-science.com/doi/abs/10.4155/ppa.14.15?journalCode=ppa

2.3. INTRODUCTION TO PEPTIDES

Peptides are short chains of amino acids linked together by peptide bonds. They are polar molecules possessing a carboxyl group at one end and an amino group at the other end.[6] Certain peptides are biologically active molecules and are capable of binding to specific biological targets in a highly selective and efficient manner.

[5]Source: https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/quaternary-structure
[6] Source: http://www.wisegeek.com/what-is-a-synthetic-peptide.htm

Functional peptides are usually modulators of cell signaling pathways, the immune system, endocrine secretions and enzymes, and are capable of regulating various important functions in the body. Their role as modulators of important biological pathways combined with their specificity has also been shown to possess tremendous pharmacological value. In addition, due to their high specificity, peptides are known to be safe and well tolerated.[7]This has greatly aroused the interest of researchers and medical professionals to develop these molecules as therapeutic products for the treatment of various diseases.

2.3.1. PEPTIDE STRUCTURE

Naturally occurring peptides are found in almost all living organisms. As indicated earlier peptides are short sequences of amino acids, which are amphoteric biological micro-molecules that contain an amino (NH2-) group, a carboxylic (-COOH) group and a side chain that is unique to each amino acid. The side chain of each amino acid confers specific properties to these biomolecules and is responsible for various interactions with other amino acids and / or other biological moieties. However, the formation of a peptide linkage between two amino acids does not involve the side chain; rather, such a bond is formed as a result of a condensation reaction (also known as a dehydration synthesis reaction) between the amino group of one amino acid and the carboxylic group of another, to form an amide bond (peptide bond).

2.3.2. CLASSIFICATION

Peptides can be broadly categorized under two categories, namely synthetic peptides and recombinant peptides. These have been explained in the sections below.

2.3.2.1. SYNTHETIC PEPTIDES

A synthetic peptide can be defined as one that is synthesized artificially using chemical methods, such as solid-phase peptide synthesis.[8] Synthetic peptides may also contain unnatural amino acids and are synthesized in laboratories.[9] These peptides resemble naturally occurring peptides, and therefore, can be used as therapeutic interventions for several chronic disorders.

2.3.2.2. RECOMBINANT PEPTIDES

Peptide drugs produced utilizing genetic engineering methods are called recombinant peptide therapeutics. Such peptide molecules are synthesized by exploiting the natural cellular machinery of microorganisms, such as bacteria, fungi, some plants and animals. It is worth

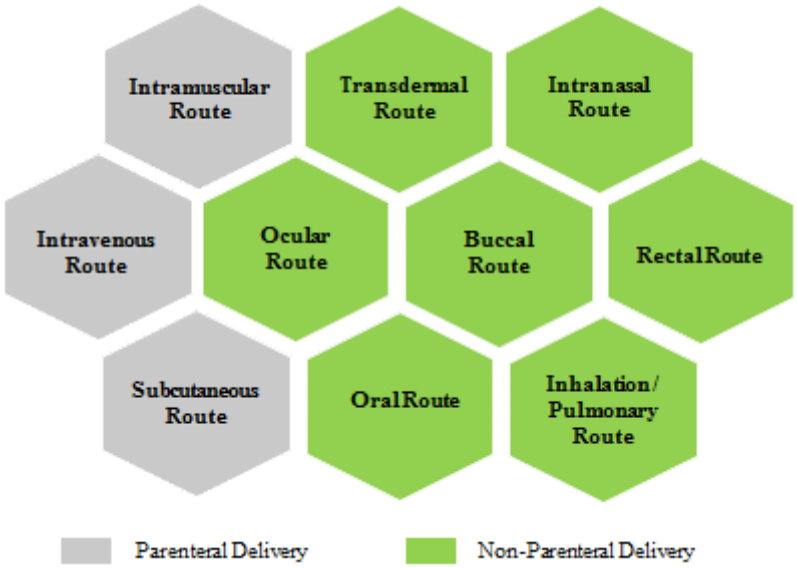
[7] Source: http://www.livestrong.com/article/248310-what-are-the-functions-of-peptides/
[8] Source: http://www.wisegeek.com/what-is-a-synthetic-peptide.htm
[9] Source: http://www.wisegeek.com/what-is-a-synthetic-peptide.htm

highlighting that most of the longer and more complex peptides are synthesized using recombinant DNA techniques.[10]

2.4. ROUTES OF DRUG DELIVERY FOR PROTEINS / PEPTIDES

Till date, there are a number of ways to administer drugs into a patient's body. For proteins / peptides, the most popular and efficient mode of administration is the parenteral route. However, to overcome the drawbacks associated with the invasive nature of parenteral administration, a number of initiatives have been launched to develop proteins / peptides that can be delivered through non-invasive pathways, such as the oral and transdermal routes. Figure 2.2 highlights various routes of administration that are being used for the delivery of proteins and peptides in the pharmaceutical industry.[11]

Figure 2.2 **Routes of Drug Delivery for Proteins / Peptides**



Source: Roots Analysis

2.5. ORAL DELIVERY OF PROTEINS / PEPTIDES

At present, protein / peptide therapeutics are mainly administered through the parenteral route (intramuscular, intravenous or subcutaneous). This is primarily due to various factors that limit the bioavailability of these drugs when administered through oral and non-oral mucosa routes. Some of the factors hindering the efficacy of such therapeutics include the following:

- Large molecular size
- Poor permeability
- Degradation in the GI tract (high sensitivity to digestive enzymes)

[10] Source: http://www.genscript.com/recombinant_pep.html
[11] Source: http://www.ddfsummit.com/wp-content/uploads/2017/09/Joel-Richard-2017.pdf

However, the oral route is associated with various advantages, such as improved patient compliance, ease of administration and cost savings.[12]Therefore, several stakeholders have shown an increased interest in the development of oral delivery of proteins / peptides.[13]

2.5.1. KEY ADVANTAGES OF ORAL DELIVERY

It has been reported that almost two thirds of pharmaceutical products are delivered through the oral route. In addition to those mentioned in the previous sections, some of the main advantages of the oral route of drug delivery are discussed below:[14]

- The delivery of oral drugs does not require a visit to the clinic or a healthcare provider to administer the drug.
- From a therapeutic perspective, oral delivery of peptides is a more physiological route as compared to injectable formats.
- The concentration of peptides in oral dosage form is comparatively higher than those delivered parenterally.
- This route of delivery does not involve the use of needles, resulting in improved patient compliance.

2.5.2. KEY CHALLENGES ASSOCIATED WITH ORAL DELIVERY

As indicated earlier, the oral administration of proteins / peptides has its own set of hurdles and challenges. Some of the key concerns regarding this route of drug deliver are briefly discussed below:[15]

- Proteins / peptides possess high molecular weights and are either hydrophilic, or lipophilic, in nature. These properties impart poor permeability characteristics to biologics and make it difficult for them to enter into cells through various biological membranes and mucosal surfaces.
- Most therapeutic proteins / peptides have short half-lives *in vivo*. This is due to the fact that these molecules are rapidly metabolized in the liver and other tissues through protein-modifying mechanisms, proteolytic enzymes and other clearance mechanisms.
- Proteins / peptides become unstable in the GI tract and intestinal lumen due to the presence of various chemical, physical and biological barriers, resulting in a significant loss of biological activity. The main function of these barriers is to protect the body from antigens, pathogens or any other harmful substances, and digest and absorb nutrients.

[12] Source: https://www.sciencedirect.com/science/article/pii/S1319016414000590
[13] Source: https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/routes-of-administration
[14] Source: http://www.pharmatips.in/Articles/Pharmaceutics/Parenteral/Advantages-And-Disadvantage-Of-Parenteral-Administered.aspx
[15] Source: https://www.sciencedirect.com/science/article/pii/S1319016414000590

CHAPTER 3

CASE STUDY: PROTEIN / PEPTIDE MANUFACTURING

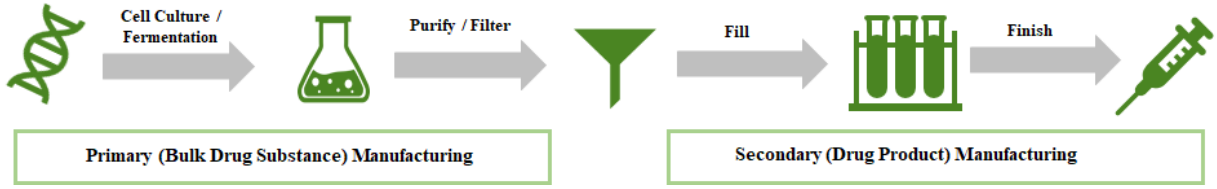
3.1. CHAPTER OVERVIEW

This chapter reviews the current state of the protein / peptide contract manufacturing market. It highlights the key steps involved in the manufacturing of proteins / peptides, along with the associated challenges. Some of the drawbacks mentioned in the subsequent sections are actually responsible for creating additional business opportunities for CMOs in the biopharmaceutical market. Further, the chapter features a detailed discussion on the various factors that need to be taken into consideration by pharmaceutical companies while selecting the right CMO partner. It also highlights certain future prospects that CMOs may need to consider in order to sustain growth in the coming years. In addition, the chapter includes a list of the leading contract manufacturers that currently claim to have the necessary capabilities to provide protein / peptide manufacturing services.

3.2. KEY STEPS INVOLVED IN PROTEIN / PEPTIDE MANUFACTURING

Proteins / peptides are biological moieties that have lately been extensively engineered / developed into therapeutics to treat a wide spectrum of disease indications. Biologic drug manufacturing essentially involves steps such as cell line selection and various upstream processing elements, followed by a fermentation process which takes around 4-10 days. It is worth highlighting that the fermentation process requires strict monitoring. Further, downstream processing is required, which involves multiple steps related to purification of the active ingredient; this involves different chromatographic separation and protein concentration methods. Post the extraction / isolation of the desired protein product, the drug substance is stabilized via an appropriate technique, such as lyophilization (freeze-drying), as required.[16] Figure 3.1 highlights the major steps involved in the manufacturing process of biologics.

Figure 3.1 **Key Steps Involved in the Manufacturing of Biologics**



Source: Roots Analysis

[16] Source: http://www.pharmabiz.com/ArticleDetails.aspx?aid=79467&sid=21

3.3. CHALLENGES ASSOCIATED WITH PROTEIN / PEPTIDE MANUFACTURING

Some of the challenges faced by product developers in this field are listed below:[17]

- Concerns related to the complexity of the manufacturing processes required to generate / produce the complex chemical structures of proteins / peptides
- Challenges associated with the maintenance of cell banks (which are often the starting point for product development and production of biologics), and sustaining batch-to-batch consistency
- Issues related to the immunogenic potential of such drugs, which is subject to change even with minor changes in the manufacturing process
- Concerns related to the huge cost of construction
- Concerns related to the high cost of raw materials required for manufacturing biologics.

3.4. CURRENT SCENARIO OF PROTEIN / PEPTIDE MANUFACTURING

Currently, only a few pharmaceutical companies have in-house manufacturing facilities and capabilities for biologics. Novo Nordisk, which claims to be a leading oral protein / peptide drug developer in this field, is one of the players that is carrying out in-house manufacturing and packaging of its lead drug candidate, oral semaglutide. at its facilities based in Clayton (US)and Malov (Denmark), respectively.[18]

Amongst the large pharmaceutical companies, Ironwood Pharmaceuticals has in-house manufacturing capabilities. Presently, the company claims to be capable of meeting the worldwide demand for its lead product, Linzess. CMOs with large capacities are further upgrading their capabilities by acquiring large scale manufacturing equipment to expand their respective businesses. Such players are also strategically increasing their geographical presences in order to acquire additional business opportunities from different global regions.

3.4.1. SELECTING A SUITABLE CMO PARTNER

Presently, the majority of pharmaceutical developers are outsourcing a range of operational activities from across the supply chain at different stages of development of their proprietary products (starting from early stage development to commercial scale manufacturing) to CMOs, in order to shorten drug development timelines and gain access to the established technical expertise and (relatively) vast infrastructure of contract service providers.[19] As indicated earlier

[17]Source: http://www.chinesepeptide.com/english/peptide-technique-support/large-scale-peptide-production.html
[18] Source: https://www.pharmaceutical-technology.com/projects/novo-nordisk-dapi-production-facility-clayton/
[19] Source: http://www.cmcbio.com/resource-center/news/advancing-biologics-development-and-manufacturing

in the report, CMOs offer a number of benefits. Some of the main reasons for selecting a CMO partner are mentioned below:[20]

- **Cost savings**: Companies that partner with CMOs need not invest in establishing new facilities, employing, training and maintaining a proper workforce. This has led several major CMOs to investigate novel strategies to cut down production costs.
- **Access to advanced capabilities and technologies**: Partnering with CMOs grants customers (drug / therapy developers) access to capabilities and technology platforms that they would otherwise have to procure at very high costs.
- **Validated quality control setup**: It has been observed that contract manufacturers have a better understanding of the manufacturing services that they offer. It is, therefore, very likely that they have stringent quality control protocols in place. Hence, it is easier for a company to rely on the expertise of a third-party manufacturer rather than establishing and validating their own processes.

Figure 3.2 **Factors for Selecting a CMO Partner**

Source: Roots Analysis

[20] Source: https://www.boundless.com/business/textbooks/boundless-business-textbook/international-business-4/types-of-international-business-41/contract-manufacturing-211-6130/

CHAPTER 4

EMERGING TRENDS ON SOCIAL MEDIA

4.1. CHAPTER OVERVIEW

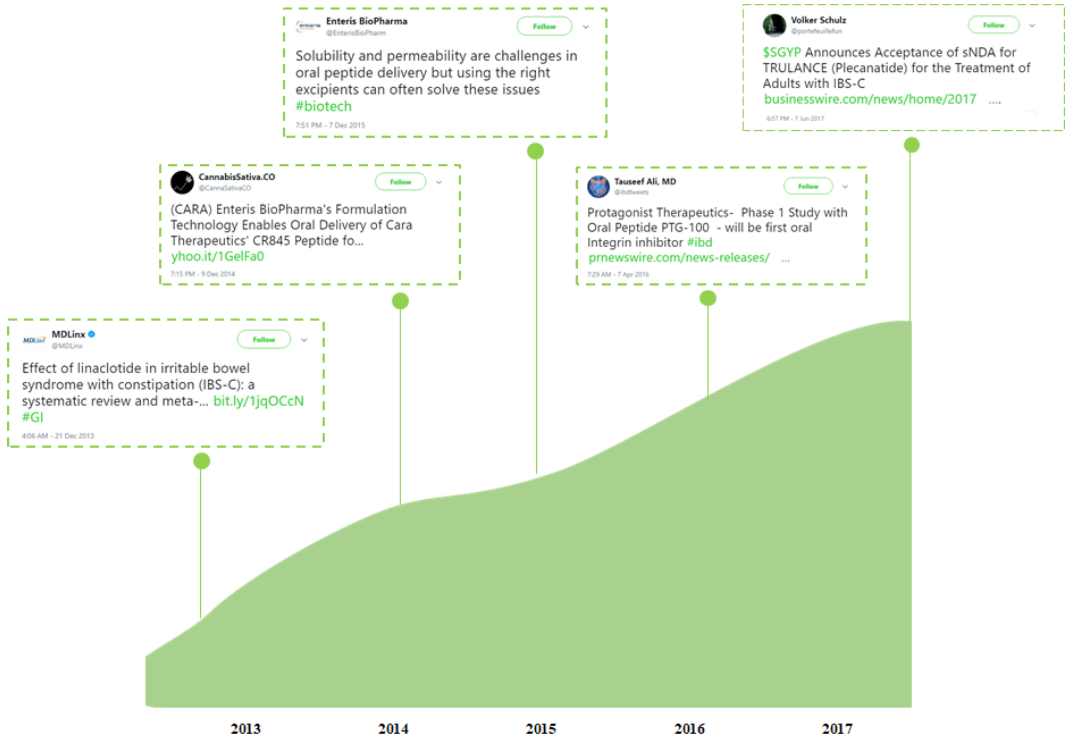
Social media has become an important platform for exchanging ideas and publicizing information on almost any topic. Within the biomedical sector, thousands of individuals follow updates posted by pharmaceutical companies on this platform. Today, many key stakeholders in the industry are also posting company updates and information on various initiatives undertaken by them, in order to keep their followers informed. Moreover, discussions about various topics and issues help drug / therapy developers recognize and become aware of patients' demands.

In this section, we have analyzed the popularity of oral proteins / peptides on the basis of the tweets posted on twitter, one of the most popular social media platforms till date. It is important to mention that we used a combination of different keywords for identifying relevant tweets for our dataset, between January 2013 and December 2017.

4.2. ORAL PROTEINS / PEPTIDES: TRENDS ON TWITTER

Figure 4.1 represents the trends on twitter in the given time period. We have also highlighted the most popular tweets within this time period.

Figure 4.1 **Oral Proteins / Peptides: Trends on Twitter (2013-2017)**



Note: Between 2013 and 2017, we were able to identify over 9800 tweets
Source: Roots Analysis; Twitter

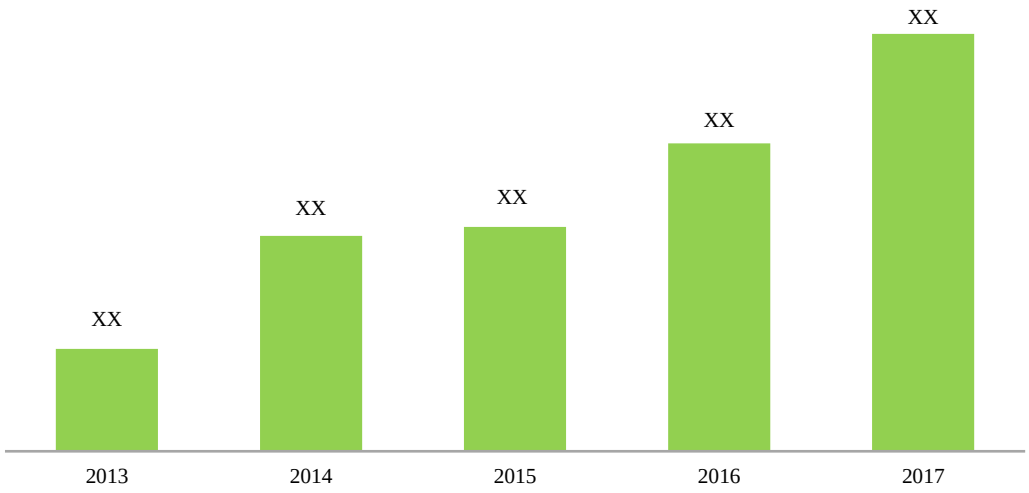
On the basis of the tweets posted on Twitter by eminent researchers and key industry players, we tried to identify the advancements and prevalent trends in the field of oral proteins / peptides in the time period between January 2013 and December 2017.

The figure clearly highlights that the number of tweets related to oral proteins / peptides have significantly increased during the given time period. There was a gradual increase in the number of tweets in the year 2017. This was primarily attributed to the approval of Trulance, a drug developed for the treatment of IBS-C in January 2017. The increasing trend in the number of tweets posted each successive year may be attributed to the surge in R&D activity in these years.

4.3. ORAL PROTEINS / PEPTIDES: YEAR-WISE ACTIVITY ANALYSIS (2013-2017)

Figure 4.2 presents the yearly volume of tweets posted between January 2013 and December 2017.

Figure 4.2 **Oral Proteins / Peptides: Year-Wise Activity Analysis by Volume of Tweets (2013-2017)**



Source: Roots Analysis; Twitter

As clearly indicated in the figure, the total number of tweets have considerably increased from 2013 to 2017.

4.4. PROTEINS / PEPTIDES: POPULAR PLAYERS ON TWITTER

Figure 4.3 highlights the key players (*in terms of the frequency of appearance in the sample dataset*) involved in the field of oral proteins / peptides in the given time period. Companies such as Ironwood Pharmaceuticals and Synergy Pharmaceuticals have been shown to frequently post updates on Twitter.

Figure 4.3 **Oral Proteins / Peptides: Popular Players on Twitter**

Source: Roots Analysis; Twitter

CHAPTER 5

FUNDING AND INVESTMENT ANALYSIS

5.1. CHAPTER OVERVIEW

In this chapter, we have reviewed the various capital investments that have been made into this field. It includes details of only those instances where investments were made into different companies / research institutes for the development of oral protein / peptide therapeutics and oral delivery technologies, offering insights on how the overall market has evolved in terms of investment activity. We have also highlighted the most active venture capital firms in this domain.

(*Please note that the content presented in this chapter is illustrative, as the complete section is the proprietary property of Roots Analysis Pvt. Ltd. and protected by the company's copyright. Additional information about the Funding and Investments is available in the full report*)

5.2. TYPES OF FUNDING

There are several ways in which a company may receive financing. For the purpose of this analysis, we have considered the following types of funding:

- **Grant:** Grants are provided by various government and non-government agencies, such as the National Institutes of Health (NIH) and the Bill and Melinda Gates Foundation, respectively. Generally, the amount invested into a company in the form of a grant is relatively less than that received through other types of funding; however, grants enable several small companies to execute very early stage investigations and bring their ideas into mainstream research and development.
- **Seed:** Seed funding is a type of an early investment that is made into a start-up to initiate their operations. The amount invested at this stage is small and is required by the company to manage its early expenses, such as those required to set up the company. It is important to mention that this is a highly risky investment for the investor.
- **Venture Capital Investments:** Venture capital investments are a type of equity financing provided by one investor or a group of investors to growing startups that are deemed to possess lucrative growth potential. In lieu of the money invested, investors acquire an equivalent stake in the company.

  Progressive rounds of venture capital funding are denoted as Series A, Series B, Series C, Series D, Series E and so on. Series A funding refers to the investment made into a company

after the seed funding round. It is worth mentioning that in each subsequent funding round, the capital invested also becomes larger, while the associated risks are relatively lower.

- **Initial Public Offering (IPO):** An IPO refers to the instance where a private company offers its stocks / shares to the public for the first time. Such an offering is usually made by small companies to fund the development of their product candidates or to monetize the investments of investors who had financed the company during its early stages.
- **Secondary Offerings:** Finances raised through all public offerings following an IPO have been captured under this category.
- **Other Equity:** All other forms of equity investments, including direct stock offerings, registered direct offering, private placement of shares and over-allotment financing that could not be classified in the categories mentioned above, have been placed under this category.
- **Debt Financing:** Debt Financing refers to those instances where a company takes a loan from either a bank or an investor / a group of investors (venture debt) and is required to pay back the money with the interest due, irrespective of whether it is in profit or not.

5.3. ORAL PROTEINS / PEPTIDES: FUNDING AND INVESTMENT ANALYSIS

Table 5.1 provides details on the funding instances that have taken place in the history *(in reverse chronological order)* of the companies engaged in the development / manufacturing of oral protein / peptide therapeutics and oral delivery technologies. For every instance represented in the table, we have mentioned the type of funding and also included the names of the investor(s), wherever available.

Table 5.1 **Oral Proteins / Peptides: Funding and Investment Analysis**[21, 22]

S. No.	Company	Month-Year	Type	Amount Invested (USD Million)	Investor(s)
1	Carmot Therapeutics	Jan-2018	Venture (Series B)	15	Horizons Ventures, The Column Group. Some private investors, including Jerome Dahan[23]
19	Gila Therapeutics	Apr-2017	Other Equity	0.76	NA[24]

[21] Information in this report (and specifically this table) has been captured from publicly available sources on a '*best-effort*' basis. However, we realize that some data points may not be publicly available and, as such, may have been overlooked in our analysis. If you'd like to notify us of these gaps, please send an email to support@rootsanalysis.com

[22] The instances in the table have been arranged in reverse chronological order

[23] Source: https://www.businesswire.com/news/home/20180117005516/en/Carmot-Therapeutics-Announces-Close-Series-Financing/?feedref=JjAwJuNHiystnCoBq_hl-RLXHJgazfQJNuOVHefdHP-D8R-QU5o2AvY8bhI9uvWSD8DYIYv4TIC1g1u0AKcacnnViVjtb72bOP4-4nHK5ieT3WxPE8m_kWI77F87CseT&utm_source=dlvr.it&utm_medium=twitter

[24] Source: https://www.crunchbase.com/organization/gila-therapeutics

S. No.	Company	Month-Year	Type	Amount Invested (USD Million)	Investor(s)
27	Aquestive Therapeutics[25]	Aug-2016	Debt Financing	50	Perceptive Advisors[26]
30	Entera Bio	Jul-2016	Debt Financing	7.5	Pontifax[27]
45	Oramed Pharmaceuticals	Dec-2015	Secondary Offering	50	Hefei Tianhui Incubator of Technologies[28]
51	Avaxia Biologics	Apr-2015	Venture (Series Unknown)	0.043	Maine Angles[29]
56	Intrexon	Jan-2015	Secondary Offering	57.5	NA
81	Rani Therapeutics	Aug-2013	Venture (Series B)	10	InCube Ventures, Google Ventures
101	Carmot Therapeutics	Jul-2012	Venture (Series A)	0.5	The Column Group[30]
122	Tarsa Therapeutics	Jul-2011	Venture (Series Unknown)	24.5	MVM Life Science Partners, Quaker BioVentures, Novo Holdings, Unigene Laboratories
154	CureDM	Aug-2009	Venture (Series Unknown)	2.1	Undisclosed[31]
169	enGene	Mar-2008	Venture (Series A)	6.4	Adams, Harkness & Hill Technology Ventures
179	Cara Therapeutics	Nov-2006	Venture (Series C)	19	MVM Life Science Partners, Alta BioPharma Partners, Ascent Biomedical Ventures[32]
189	Nutrinia	Jun-2005	Venture (Series A)	1	New Generation Technologies[33]

Source: Roots Analysis

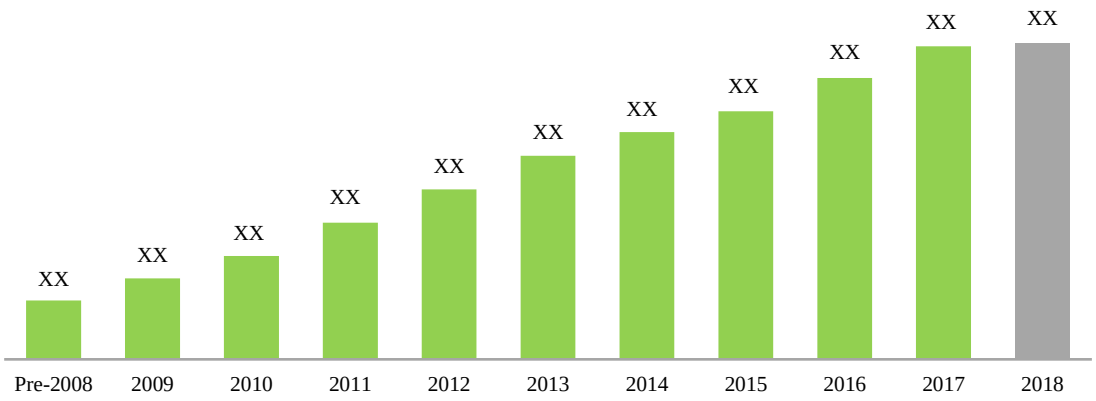
5.3.1. Analysis by Cumulative Number of Funding Instances

In the recent years, oral proteins / peptides have garnered substantial interest from several venture capital firms. As illustrated in the figure, the number of funding instances in this domain have been increasing at a healthy rate. In fact, in 2017 alone, we identified 20 instances of funding.

Figure 5.1 presents a cumulative representation of the yearly trend of investments made in the period between 2008 and 2018 (till January).

[25]Source: https://www.prnewswire.com/news-releases/monosol-rx-changes-name-to-aquestive-therapeutics-and-expands-cns-product-portfolio-300564299.html
[26]Source: https://www.cnbc.com/2016/08/17/globe-newswire-monosol-rx-closes-50-million-credit-facility-with-perceptive-advisors.html
[27]Source: http://www.globes.co.il/en/article-entera-bio-raises-75m-1001141522
[28]Source: http://nocamels.com/2015/12/oramed-receives-50m-investment-from-largest-chinese-pharmaceutical/
[29]Source: https://search.wellspringsoftware.net/organization/avaxia-biologics
[30]Source: https://www.cbinsights.com/deal/carmot-therapeutics-series-a-ii
[31]Source: https://www.crunchbase.com/organization/curedm
[32]Source: http://ir.caratherapeutics.com/releasedetail.cfm?ReleaseID=807855
[33]Source: https://www.crunchbase.com/funding_round/nutrinia-series-a--8c6dfbd7

Figure 5.1 Oral Proteins / Peptides: Cumulative Number of Funding Instances, Pre-2008-2018

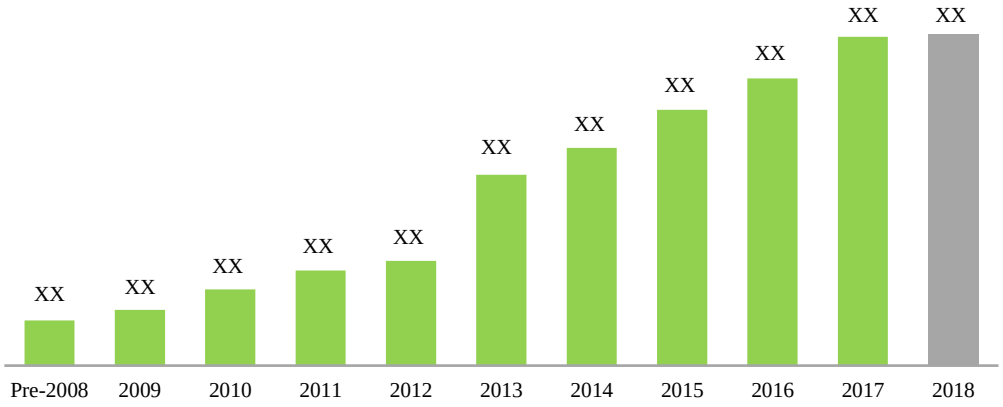


Note: For 2018, funding instances have been captured till January
Source: Roots Analysis

5.3.2. Analysis by Cumulative Amount Invested

Figure 5.2 depicts the cumulative amount invested (in USD million) in the given time period.

Figure 5.2 Oral Proteins / Peptides: Distribution of Amount Invested by Year, Pre-2008-2018 (USD Million)



Note 1: For 2018, funding instances have been captured till January
Note 2: Cases where the amount invested was not disclosed have not been included in this analysis

Source: Roots Analysis

The growing number of funding instances and increasing capital amounts invested are indicative of the enormous potential that experts believe resides within this domain, which has managed to capture and retain the interest of several venture capitalists and other investors.

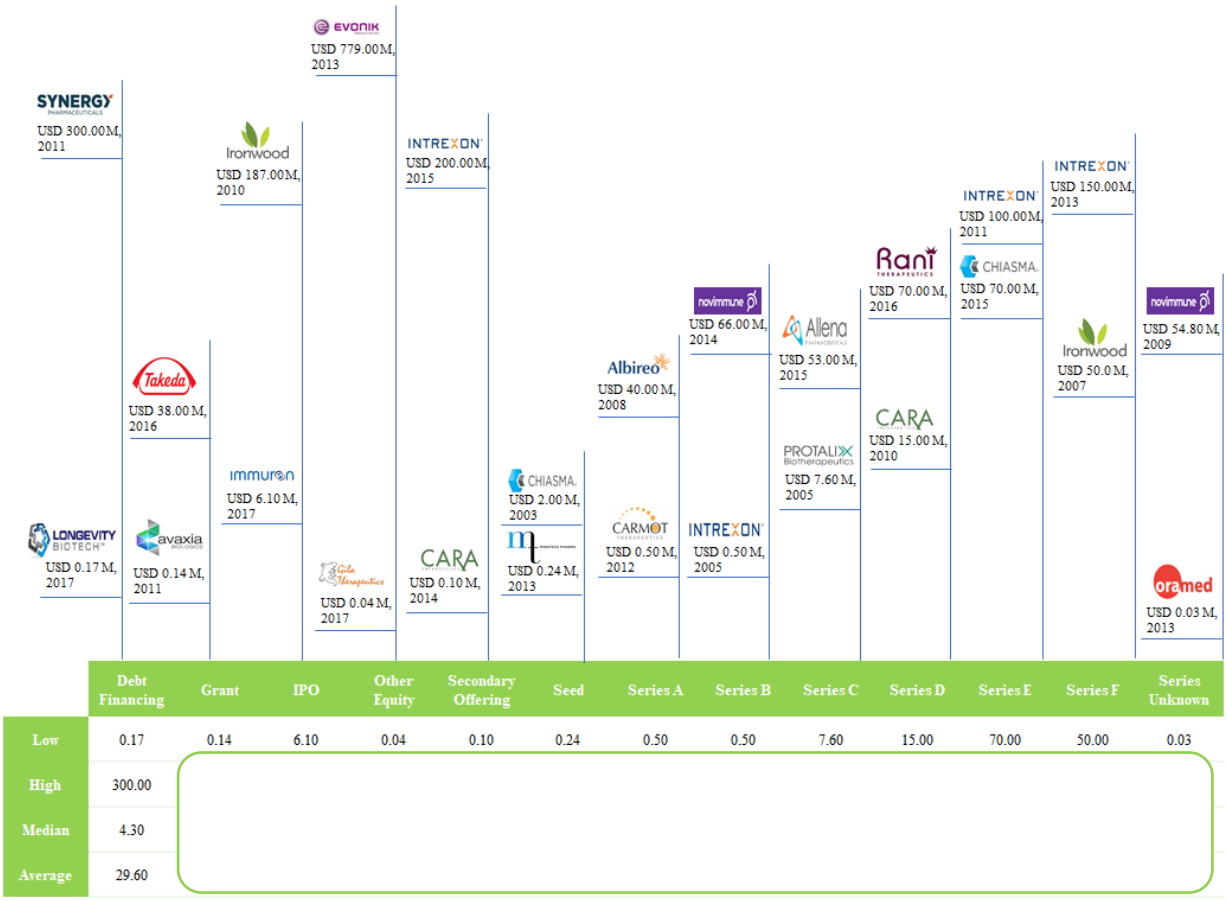
5.4. Concluding Remarks

The analysis presented in the earlier section clearly demonstrates that the interest of venture capitalists in the space of oral proteins / peptides has increased at a healthy rate over the last

couple of years. In the funding instances mentioned in table 5.1, a significant amount of variation was observed within a particular category of financing.

Figure 5.3 provides a pictorial summary of the investments made within this domain, highlighting maximum, minimum and mean amounts invested within each category described above. It is worth noting that in the figure we have presented only those categories for which more than two instances were observed.

Figure 5.3 **Oral Proteins / Peptides: Funding and Investment Summary**



	Debt Financing	Grant	IPO	Other Equity	Secondary Offering	Seed	Series A	Series B	Series C	Series D	Series E	Series F	Series Unknown
Low	0.17	0.14	6.10	0.04	0.10	0.24	0.50	0.50	7.60	15.00	70.00	50.00	0.03
High	300.00												
Median	4.30												
Average	29.60												

Note: The funding type Venture (Series G) have been excluded from this analysis since it had only one funding instance
Source: Roots Analysis

VECTOR MANUFACTURING MARKET: PARTNERSHIPS AND COLLABORATIONS

CHAPTER 6

INTRODUCTION TO VECTOR MANUFACTURING

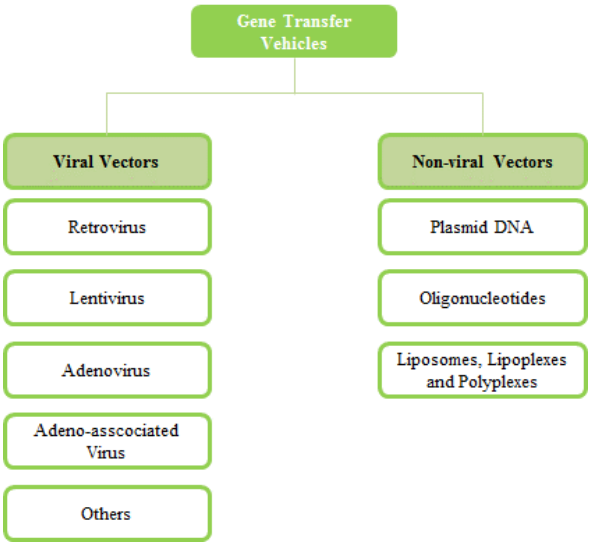
6.1. CHAPTER OVERVIEW

This chapter provides detailed introduction and information on classification of the different types vectors on the basis of multiple parameters. Further, it goes on to discuss why viral vectors are preferred for gene delivery, along with a detailed historical background related to such gene delivery vehicles. The chapter also provides details on the various methods of transfection used to facilitate gene delivery using non-viral vectors. Additionally, it includes elaborate details of the applications of vectors based on the type of therapy and the therapeutic area for which they are being evaluated.

6.2. VIRAL AND NON-VIRAL METHODS OF GENE TRANSFER

As indicated earlier, vectors may be based on viruses or other molecular methods that enable gene delivery. Over the last few decades, various viral and non-viral vectors have been optimized and standardized for the purpose of gene delivery. Figure 6.1 provides a list of the various types of viral and non-viral gene vectors.

Figure 6.1 **Gene Transfer: Viral and Non-Viral Methods**



Note: Other vectors include alphavirus, foamy virus, herpes simplex virus, Sendai virus, simian virus and Vaccinia virus

Source: Roots Analysis

Although non-viral vectors are usually less efficient than viral vectors, they offer a number of advantages, including low immunogenicity and a large packaging capacity (for therapeutic DNA

molecules).[34] However, there are efforts underway to add certain viral characteristics, specially related to receptor mediated uptake and nuclear translocation of DNA, in order to improve non-viral gene transfer methods.

6.3. TYPE OF VIRAL VECTORS

Using viruses as vectors involves the manipulation of viral genome; essentially all virulence genes need to be removed (to prevent viral infection) and replaced with a functional copy of a therapeutic gene(s), along with all the necessary regulatory sequences that control its expression. These modified viruses are able to carry the specific target cells with high efficiency. Certain features that need to be considered for using viruses as therapeutic tools are highlighted below:

- **Safety:** Viral vectors are based on pathogenic organisms; therefore, they need to be significantly modified (at the genetic level) in order to minimize handling and post-treatment risks. In fact, the part of the viral genome that is responsible for its replication is usually removed. This allows the virus to infect a patient's cells and deliver the gene of interest, without replicating.
- **Stability:** Viruses that are not genetically stable and can quickly rearrange their genomes should not be used to develop vectors. To ensure stability, DNA replication involves a proof reading step, unlike RNA replication. Therefore, the use of RNA based viral vectors are prone to develop unwanted mutations, which may be detrimental to the host.
- **Cell type specificity:** Owing to advances in molecular manipulation technologies, viral vectors can be designed to target either a specific kind of cell, or a wide range of cells within the body of a host. Such a process involving construction of viruses or viral vectors in combination with foreign viral envelope proteins is known as pseudotyping.
- **Selection capability:** Transduced cells are usually isolated with the help of certain selectable markers that are incorporated into the viral vectors. The most commonly used selectable markers are antibiotic resistance genes.
- **Low immunogenicity:** In the case of *in vivo* gene therapies, the immunogenicity of a viral vector can impact the efficacy, and stability, of gene transfer. Therefore, viral vectors should be modified in such a way that the patient's body does not develop an immune response against them.

6.3.1. ADENO-ASSOCIATED VIRAL VECTORS

6.3.1.1. OVERVIEW

[34] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507026/

Adeno-associated virus (AAV) is a small-sized virus of the *Parvoviridae* family that has a single stranded DNA genome. This virus is capable of infecting a broad range of host cells, including both dividing and non-dividing cells. In addition, it is a non-pathogenic virus that does not generate an immune response in most patients.

The AAV genome comprises of inverted terminal repeats (ITRs) at both ends of the DNA strand and two ORFs, namely rep and cap. Each ITR sequence consists of 145 bases that have the ability to form a hairpin structure. These sequences are required for the primase-independent synthesis of a second DNA strand and the integration of the viral DNA into the host cell genome. The rep genes encode proteins that are required for the AAV life cycle and site-specific integration of the viral genome. Cap genes encode the capsid proteins, namelyVP1, VP2 and VP3.[35]

6.3.1.2. ADVANTAGES

Over the last few years, AAV vectors have emerged as an extremely useful and promising mode of gene delivery. Some of the advantages of these viral vectors are listed below:

- Possess characteristics that allow efficient manipulation of the vector (as required).
- Possess the ability to be easily purified, as they are not readily degraded by shear forces, enzymes or solvents.
- Exhibit reduced risk of adverse inflammatory reactions, due to non-pathogenic nature and less immunogenic properties.
- Allow delivery of genetic sequences of up to ~4 kb.[36]
- Exhibit reduced risk of ectopic integration of the therapeutic DNA.

6.3.1.3. LIMITATIONS[41]

The major drawbacks of these viral vectors are as follows:

- Lack the capability to deliver larger amounts of genetic material (than what is mentioned above).
- Characterized by low transduction efficiencies, due to the need for second strand synthesis for the generation of double stranded DNA that is required for gene expression.[37]

(*Please note that the content presented in this section is illustrative, as the complete section is the proprietary property of Roots Analysis Pvt. Ltd. and protected by the company's copyright. Additional information about the various vector types is available in the full report*)

[35] Source: http://www.genetherapynet.com/viral-vector/adeno-associated-viruses.html
[36]Source: http://files.shareholder.com/downloads/AMDA-2H6BI7/1891876449x0xS1564590-15-7858/1273636/filing.pdf
[37]Source: http://www.cellbiolabs.com/news/adeno-associated-virus-aav-provides-advantages-gene-delivery

6.3.2. OTHER VIRAL VECTORS

6.3.2.1. ALPHAVIRUS

Alphaviruses belong to the *Togaviridae* family of viruses. These are capable of infecting both vertebrates and invertebrates. The alphavirus genome is a single stranded RNA molecule, which is typically 11 to 12 kb, having a 5' cap and 3' poly-A tail. In alphaviruses, the expression of viral proteins and the replication of the viral genome takes place in the cytoplasm of the host cell. It is worth mentioning that certain retroviral and lentiviral vectors are usually pseudotyped using alphavirus envelope proteins, which facilitate the recognition and infection of a wide range of potential host cells.[38]

(*Please note that the content presented in this section is illustrative, as the complete section is the proprietary property of Roots Analysis Pvt. Ltd. and protected by the company's copyright. Additional information about the other viral vector types is available in the full report*)

6.4. TYPES OF NON-VIRAL VECTORS

Viral vectors are known to cause inflammation, invoke immunological responses and in certain cases, lead to non-specific transduction. These limitations can be circumvented by using non-viral gene delivery approaches. It has been shown that therapeutic genes can be introduced into a host by a number of non-viral methods as well. This form of gene transfer may involve either a direct approach, such as the injection of naked DNA into a cell, or an indirect approach, which involves enclosing the therapeutic gene within a carrier complex. In both these cases, therapeutic gene sequences are inserted into a plasmid along with all the necessary sequences required for its expression within the host.

Further, non-viral vectors have limited immunogenicity that makes them safe and allows re-dosing without the risk of any significant complications. They are economical as they be easily produced in large quantities. Some of the commonly used non-viral gene delivery methods have been discussed in the following sections.

6.4.1. PLASMID DNA

Plasmid vectors are one of the most common non-viral gene delivery tools that are used to insert transgenes into target cells. Basically, plasmids are small circular segments of extrachromosomal DNA that are found in bacteria. Isolation of these plasmids from bacteria can be carried out by enzymatic or mechanical means, followed by affinity separation. Plasmids can be easily modified to deliver a therapeutic gene and optimize its expression in a host cell. The

[38]Source: http://www.genetherapynet.com/viral-vector/alphaviruses.html

incorporation of a multiple cloning site into a plasmid enables the insertion of a gene of interest with the help of restriction enzymes. The circular DNA is nicked at the multiple cloning site and, after the incorporation of the gene of interest, the nicks are annealed via a ligation step. The gene present in these vectors is generally flanked by a promoter sequence and a transcription terminator sequence to facilitate proper expression after it is incorporated into the host genome. Plasmids with inducible promoters are widely preferred as gene delivery tools, owing to the fact that these vectors offer the flexibility to activate / deactivate gene expression as required.[39]

6.4.2. OTHER NON-VIRAL VECTORS

Despite its many clinical benefits significance, plasmid DNA vectors are associated with certain inherent limitations; some of which are listed below:[40]

- Requirement of physical forces, vehicles or specialized modifications to facilitate cellular uptake and nuclear localization; these methods are known to often disrupt the plasmid, thereby, reducing its overall efficiency
- Dependence on antibiotics and affiliated resistance genes for the plasmid preparation process
- Presence of bacterial sequences in plasmids that may lead to gene silencing

To address these limitations, various modified versions of plasmid DNA vectors have been developed; these include minicircles and minivectors, which are briefly described below.

Minicircles: Minicircles are small excised, circular DNA fragments that are essentially obtained from a plasmid molecule. These non-viral, episomal minicircle, gene expression cassettes are usually devoid of any bacterial DNA sequences and are present in a variety of promoter and reporter combinations. Unlike standard-sized plasmids, the small size of minicircles enable more efficient transfection.

Minivectors: Similar to minicircles, these are small-sized, non-viral DNA vectors that are developed from a parent plasmid via site-specific recombination. These vectors can be modified to achieve sizes of ~350 bp (the smallest minicircle DNA is ~650 bp) and give high yields. Such vectors typically encode only for the genetic payload and short integration sequences.

[39] Source: http://biotech.about.com/od/proteintechnology/g/Plasmids.htm
[40] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5333054/

6.4.3. METHODS OF GENE DELIVERY USING NON-VIRAL VECTORS: METHODS OF TRANSFECTION

The process of introducing nucleic acids into target cells is called transfection. The term is primarily used in reference to the introduction of non-viral vectors into cells. This can be brought about using a variety of different techniques. Some of them are described briefly in the following sections.

6.4.3.1. BIOLISTIC METHODS

The bioballistic, or biolistic, method is the simplest method of transfection. Although it was originally designed for targeted gene delivery in plants, of late, this method has shown significant promise as a gene delivery system in higher mammals. A gene gun, which is the tool used in this type of gene deliver, employs the use of microscopic gold or silver particles coated with multiple copies of the therapeutic DNA. These particles are accelerated to high velocities by using compressed helium, or a high voltage electric discharge, and shot across the plasma membrane directly into the cell.

6.4.3.2. ELECTROPORATION

Electroporation involves the use of an electrical field to create transient pores in the cell membrane to facilitate the entry of impermeable macromolecules into the cytoplasm. Hydrophilic molecules, such as DNA, RNA and proteins, can easily enter into the cell through these pores. Although this technique was initially developed to transfer DNA into bacteria, yeast and mammalian cells *in vitro*, it has only recently been applied to living animals as well. So far, this method had been successfully used to deliver transgenes into skeletal muscles, liver tissue, cardiac tissue, skin, cells of the vasculature, cornea cells and kidney cells. It has been proven that the use of electroporation results in a 100-1,000-fold increase in gene expression as compared to DNA injection.

6.4.3.3. RECEPTOR MEDIATED GENE DELIVERY

Amongst the many procedures that are currently being developed for targeted gene delivery, receptor mediated endocytosis comes closest to fulfilling this requirement. This method exploits the natural physiological process of receptor mediated endocytosis (RME) to deliver genetic material to specific cells. For this, an antibody or ligand, which binds specifically to a cell surface receptor that is known to undergo endocytosis, is used. The ligand is covalently linked to the DNA through a polycationic adjunct, such as polylysine. These ligand-polylysine-DNA complexes retain their binding specificity to the cell surface and are taken up into the cell, where they enter the endosomal compartments through normal endocytotic processes.

6.4.3.4. GENE ACTIVATED MATRIX (GAM)

Biomaterial scaffolds have been shown to be able to act as viable templates for tissue formation, which can be used for the treatment of extensive tissue or organ damage. Interactions between the scaffold and infiltrating cells in the presence of various growth factors is intrinsic to the success of this method. However, the use of proteinaceous factors in such an environment is limited by several issues, such as the requirement of large doses, the need for repeated application, poor distribution, high cost, short half-life and protein instability.

CHAPTER 7

EMERGING VECTORS

7.1. CHAPTER OVERVIEW

The current vector manufacturing market is heavily dominated by viral vectors, such as those based on AAV, adenovirus, lentivirus and retrovirus, and certain non-viral vectors, such as those based on plasmid DNA, as well. During our research, we came across a few relatively new types of vectors that are presently being researched for the development of various therapies that require genetic modification; in this report, these upcoming vector products have been categorized as emerging vectors. The emerging vectors discussed in this chapter include vectors based on alphavirus, *B. longum*, *Listeria monocytogenes*, myxoma virus, Sendai virus and Sleeping Beauty transposon based non-viral vectors.

7.1.1. ALPHAVIRUS BASED VECTORS

Alphaviruses belong to the *Togaviridae* family. These viruses are capable of infecting both vertebrates and invertebrates. The alphavirus genome is a single stranded RNA, which is typically 11 to 12 kb, having a 5' cap and 3' poly-A tail. The genome comprises of two ORFs that code for non-structural and structural components. The non-structural ORF codes for RNA transcription and replication proteins and the structural ORF codes for the capsid and envelope proteins, such as capsid protein C, envelope glycoprotein E1, envelope glycoprotein E2 and envelope glycoprotein E3. As is the case with most viruses, the expression of viral proteins and replication of the viral genome takes place in the cytoplasm of the host cell.

We came across several companies that have the capabilities to manufacture alphavirus-based vectors or possess the necessary technology platforms to support their development / production, and / or are engaged in the development of therapies / vaccines based on these vectors. For example, Alphavax, a US based company, has several vaccines that are under development, based on these vectors. SAFC / BioReliance claims to be capable of manufacturing such vectors across all scales of operation (laboratory, clinical and commercial). On the other hand, and AlphaVax and MaxCyte Therapeutics possess technological platforms for the development / production of such vectors.[41, 42, 43]

[41] Source: http://assets.sial.com/deepweb/assets/bioreliance/marketing/documents/pdf/a/l/bioreliance_pdfs/RDI_F-1230114_GeneTherapy_Capability_Flyer_final/RDI_F-1230114_GeneTherapy_Capability_Flyer_final.pdf
[42] Source: https://www.alphavax.com/technology-overview.html
[43] Source: https://www.maxcyte.com/technology/scalability/

7.1.2. BIFIDOBACTERIUM LONGUM (B. LONGUM) BASED VECTORS

B. longum belongs to a genus of non-pathogenic, anaerobic bacteria, which is generally present in the lower small intestine and large intestine of humans and a few other mammals. The bacterium has been shown to be capable of specifically delivering a particular gene of interest to various tumor types. The bacterium is currently being utilized by Anaeropharma Science for the development of two gene therapy candidates that are in phase I / II (APS001F) and preclinical (unnamed) stages of development.[44]

7.1.3. CYTOMEGALOVIRUS (CMV) BASED VECTORS

Human cytomegalovirus (CMV) is a β-herpesvirus that has a large DNA genome (236 kb), which is known to mediate life-long, asymptomatic viral infection in healthy individuals.[45] The capability of this virus to induce T-cell responses has captured the interest of biopharmaceutical developers, prompting them to use this virus to generate vectors that could in turn be used to design and produce vaccines.

AlphaVax, Hoopika Biotech and VBI Vaccine are presently engaged in the development of certain vaccines, namely AVX601 (phase I / II), HB-101 Vaxwave (phase II) and VB-1501A (phase I), respectively, using CMV vectors. Multiple academic players, such as The Jarvis Lab, and Vaccine and Gene Therapy Institute (Oregon Health and Science University), are also exploring the potential of CMV vectors for the development of vaccines.[46]

7.1.4. LISTERIA MONOCYTOGENES BASED VECTORS

Listeria monocytogenes, a gram-positive, facultative intracellular parasite, is known to cause meningitis in immunocompromised individuals. This bacterium is generally consumed by macrophages and other phagocytic cells, in the spleen and in liver Kupffer cells. For the treatment of cancer, *Listeria monocytogenes* vectors have been shown to be a promising tool for delivering DNA, RNA or protein to cancer cells, or for priming immune responses against certain tumor-specific antigens.

Advaxis is developing six gene therapy candidates using its proprietary Lm Technology, which is based on *Listeria monocytogenes*. Aduro Biotech has three gene therapies, namely ADU-214 / JNJ-64041757, ADU-741 / JNJ-64041809 and pLADD, in phase I clinical trials, amongst

[44] Source: http://www.anaeropharma.co.jp/aps001f/
[45] Source: https://onlinelibrary.wiley.com/doi/pdf/10.1111/imm.12829
[46] Source: http://www.thejarvislab.com/research/

which, ADU-214 / JNJ-64041757 and ADU-741 / JNJ-64041809, are being developed in collaboration with Janssen Pharmaceutica.[47,48]

7.1.5. MYXOMA VIRUS BASED VECTORS

Myxoma virus is an oncotropic poxvirus that infects rabbits. It has also been shown to efficiently infect different types of mouse and human cancer cells. Moreover, the rabbit-specific pathogen can actually be used safely as a therapeutic agent in all non-rabbit hosts. Despite its narrow pathogenicity, it can replicate in a diverse range of cultured cells from several species, including a wide range of human cancer cells that are permissive to the virus. Furthermore, it has also been shown to selectively infect tumors in human xenograft models and primary mouse tumor models.[49]

DNAtrix is developing MYX-135, a novel oncolytic immunotherapy, in preclinical stage of development for the treatment of hematological malignancies. It can be anticipated that as the therapy candidate demonstrates positive results and enters clinical stage of development the popularity of this vector type is likely to increase for the development of other genetically modified therapies.

7.1.6. SENDAI VIRUS BASED VECTORS

Sendai virus, a non-segmented negative strand RNA virus, belongs to the *Paramyxoviridae* family of viruses. It was discovered in 1953 in Japan and since then, has been widely utilized in research in the field of cell biology and for various industry applications. However, its utility as a recombinant viral vector was identified recently. Its unique characteristics, which include its capacity for gene expression, low pathogenicity, and broad host range, enables scientists / developers to use this vector for the transfection of various types of animal cells. Its ability to mediate cytoplasmic gene expression makes it suitable for various applications, however, in such cases, the integration of exogenous genes may prove to be disadvantageous.[50] ID Pharma and Sanofi are currently manufacturing Sendai virus-based vectors for clinical or / and commercial scale purposes.[51]

7.1.7. SLEEPING BEAUTY TRANSPOSONS

Sleeping Beauty is a transposon based non-viral gene delivery system that combines the favorable characteristics of viral vectors (such as long-lasting transgene expression and stable chromosomal integration) with those of non-viral delivery systems (such as enhanced safety

[47] Source: http://www.aduro.com/pipeline/
[48] Source: http://www.aduro.com/technology/ladd/
[49] Source: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0109801
[50] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3504922/
[51] Source: https://www.the-scientist.com/?articles.view/articleNo/45422/title/iPSCs-and-Cancer-Risk/

profile, lower immunogenicity, and reduced GMP manufacturing costs). The vector is capable of permanently integrating with the genomic material of cells, and hence, can exhibit efficient and sustained expression. Furthermore, unlike viral vectors, transposon-based vectors can be maintained and propagated as plasmid DNA. Hence, these vectors can be manufactured at lower costs, using less sophisticated methodologies.

This vector platform is presently being utilized by several pharmaceutical players, namely ZIOPHARM Oncology, Intrexon and Merck Serono, in collaboration with MD Anderson Cancer Center for the development of CAR-T cell therapies.[52] Furthermore, the vector is commercialized by pharmaceutical players, such as Addgene, ImmunoGenes, and B-MoGen Biotechnologies.[53, 54, 55]

[52] Source: http://info.evaluategroup.com/rs/607-YGS-364/images/epv-cart16.pdf
[53] Source: https://www.addgene.org/search/advanced/?q=sleeping+beauty
[54] Source: http://www.immunogenes.com/content.php?c=Sleeping-Beauty&id=3
[55] Source: http://www.bmogen.com/cmv-egfp

CHAPTER 8

DRIVERS AND CHALLENGES

8.1. CHAPTER OVERVIEW

Owing to our exhaustive research, we could track irregularities in the current approaches for manufacturing viral vectors and plasmid DNA. In this chapter, we have focused on such irregularities and provided details on the parameters that are expected to drive the market of viral vectors based on different viruses (such as AAV, adenovirus, lentivirus or retrovirus) and plasmid DNA vectors. Furthermore, we have highlighted the challenges faced during production of these vectors. Additionally, we have provided insights offered by players active in this domain that was collated through primary research.

(*Please note that the content presented in this chapter is illustrative, as the complete section is the proprietary property of Roots Analysis Pvt. Ltd. and protected by the company's copyright. Additional information about the drivers and challenges is available in the full report*)

8.2. VIRAL VECTORS AND PLASMID DNA: DRIVERS AND CHALLENGES

8.2.1. AAV VECTORS

Over the years, there has been a notable surge in the use of rAAV vectors in genetically modified therapies. The approval of Luxturna (Spark Therapeutics) in the US and the fact that there are multiple AAV based late stage drugs, such as BMN 270 (BioMarin Pharmaceutical), AVXS-101 (AveXis, REGENXBIO) and GS010 (GenSight Biologics), in the pipeline are indicative of the therapeutic potential and the growing interest of therapy developers in this field.

Waisman Biomanufacturing is also pursuing the development of a suspension-based manufacturing process to meet certain scale-related requirements. The company claims to be capable of producing clinical batches of AAV at the 3 L–250 L scale.[56] Vector manufacturers have also reported using the baculovirus system for the production of AAV vectors. For example, Glybera, which is based on AAV1, was manufactured using the Sf9 / baculovirus system.[57]

AAV vectors are small in size and are not generally used in cases where large amounts of genetic material have to be delivered. Further, scale-up techniques for such processes depend on the use of large bioreactors, which require heavy investments and other resources. There are certain CMO facilities that offer more scalable options, involving the use of suspension cell lines in WAVE and stirred tank reactors, to help fulfill late clinical and commercial stage requirements.

[56] Source: http://gmpbio.org/clinical-production/viral-vectors-vaccines/
[57] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4755504/#bib1

Figure 8.1 presents the major challenges and drivers associated with the production of AAV vectors.

Figure 8.1 **AAV Vectors: Drivers and Challenges**

Drivers

- Currently, there are several AAV vector manufacturers in the market (~90 as per our research)
- Many pharmaceutical companies / CMOs have commercial scale capacity for vector production (~20 as per our research)
- They are suitable for *in vivo* applications due to low their immunogenicity
- Use of cell lines, such as HeLa and BHK-21, have been shown to offer to higher yields per batch run
- Scalable downstream processes, such as chromatography, can be employed to obtain pure vectors
- Use of WAVE bioreactors and stirred tank bioreactors for large scale production of vectors

Challenges

- The small size of these vectors limits the size of therapeutic gene that can be delivered
- Conventional production methods (which are used by several organizations), using HEK293T adherent cells in presence of serum, are not scalable
- Scale-up techniques require sufficient man power, space and investments that many R&D institutes / small companies do not currently have
- Construction of stable cell lines for vector production purposes, is cumbersome

Source: Roots Analysis

8.2.2. PLASMID DNA

A significant proportion of gene-modified therapy candidates require naked / plasmid DNA to transfer therapeutic genes. Plasmid DNA, as a gene delivery vehicle, has been show to demonstrate low immunogenicity and a better safety profile compared to that of viral vectors; however, their primary limitation is related to transfection efficiency, which is comparatively low in case of these vectors.[58] Furthermore, biological barriers, such as endosomal attack, renal clearance and degradation by serum endonucleases, limits the application of plasmid DNA as a gene delivery vehicle.[59] It is important to note that plasmid DNA carries a bacterial origin of replication and an antibiotic resistance gene. Therefore, such a gene delivery vehicle is associated with the risk of uncontrolled dissemination of the therapeutic gene and the antibiotic resistance gene.[60, 61]

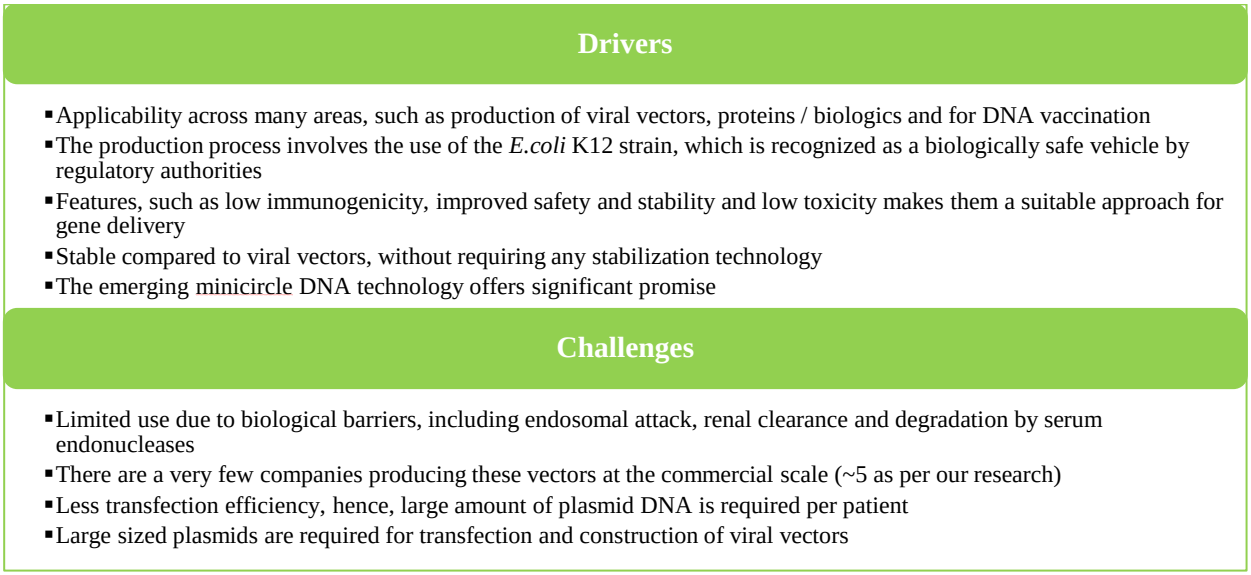
The GMP production of plasmids is a time-consuming process and takes around 4-9 months.[62] Typical plasmid DNA production processes yield 0.2 g – 2.1 g of plasmid per litre of culture

[58] Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347098/
[59] Source: http://www.bioprocessintl.com/2016/emerging-platform-bioprocesses-for-viral-vectors-and-gene-therapies/
[60] Source: http://www.bioprocessintl.com/2016/emerging-platform-bioprocesses-for-viral-vectors-and-gene-therapies/
[61] Source: https://www.nature.com/articles/3300540
[62] Source: http://vgxii.com/OEtilxPmQG5trqR/VGXI_How_Long_Does_It_Take.pdf

media. For instance, Boehringer Ingelheim's CMO division, BioXcellence, claims to be capable of generating fermentation titers of up to 3.2 g/l.[63]

Figure 8.2 presents the major challenges and drivers associated with production of plasmid DNA.

Figure 8.2 **Plasmid DNA: Drivers and Challenges**



Source: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1574-6968.2006.00594.x, Roots Analysis

It is worth highlighting that contract manufacturers, such as Cobra Biologics and Aldevron, have developed proprietary platforms (ORT technology and GMP-Source plasmid DNA, respectively) for the production of stable and high quality plasmids at large scales.[64] Further, Eurogentec announced having worked on a project that involved the production of the largest batch of GMP plasmid DNA (150 g of plasmid DNA in a single batch).[65]

[63]Source:http://www.bioxcellence.com/content/dam/internet/topical/bioxcellencenew1/com_EN/documents/BioXcellence_Setting-the-standard-for-plasmid-DNA-production_whitepaper.pdf
[64]Source: http://www.cobrabio.com/Services/DNA/Molecular-Biology
[65]Source: http://www.eurogentec.com/news/287-150-g-of-gmp-plasmid-dna.html

CHAPTER 9

PARTNERSHIPS AND COLLABORATIONS ANALYSIS

9.1. CHAPTER OVERVIEW

This chapter presents insights on collaborations specific to viral vectors and plasmid DNA. We have presented information on only those collaboration agreements that have been inked between January 2015 and February 2018. The chapter provides details on the different types of partnership models that have been adopted by players in this domain and features a comprehensive analysis of the partnership instances based on multiple parameters, such as the year of partnership, type of partnership model, geography of the collaborators, type of vector and scale of operation. In addition, the chapter provides an overview of the level of activity among the players engaged in this market.

9.2. PARTNERSHIP MODELS

The classification criteria used for the various types of partnerships considered in this analysis is described below:

- **Manufacturing Agreement:** This includes instances where one company opted to recruit the services of another company for manufacturing purposes only. Agreements with contract manufacturing organizations (CMO) for such purposes have also been considered under this category.
- **Product / Technology Licensing:** This category includes instances where one company collaborated with a vector manufacturing company solely to utilize their technology for the production of vectors.
- **Product Development:** In the product development model, a company involved in the development and / or production of a vector-based drug / therapy collaborates with other players to utilize their vector manufacturing technology / platform for use in its product candidate.
- **Process Development / Optimization:** This incorporates the agreements, wherein the companies partnered to design manufacturing processes / platforms or optimize their current processes in order to scale-up the production output.
- **Merger / Acquisition:** This category represents all those instances when one company acquires all the assets of another company and cases where two companies merge to function as a single entity.
- **Service Alliance:** This category includes those instances where two companies partnered to combine their services related to vector manufacturing. In such cases, the collaborating

companies utilize their specific capabilities, such as manufacturing capabilities, R&D services, technology and process development, for offering vector manufacturing services to third-parties.

- **Production Asset / Facility Acquisition:** This category comprises of instances where one company obtained either production assets, such as technologies or manpower, or one or more manufacturing facilities of another company.
- **Distribution Agreement:** These deals are inked with the purpose of distributing the manufactured vector products (such as viral vectors or plasmid DNA) in one or more regions.

9.3. VIRAL VECTORS AND PLASMID DNA MANUFACTURING: RECENT COLLABORATIONS AND PARTNERSHIPS

Table 9.1 provides the list of partnerships that were signed in the time period January 2015 till February 2018.

Table 9.1 Viral Vectors and Plasmid DNA: List of Partnerships[66]

S. No.	Company Name	Month-Year	Partner(s)	Vector Type	Scale of Manufacturing [67]	Nature of Collaboration
8	BioReliance (acquired by Merck)	Dec-2017	bluebird bio	Lentiviral	Commercial	Manufacturing[68]
15	Oxford BioMedica	Aug-2017	Cell and Gene Therapy Catapult, Stratosphase, Synthace	Lentiviral	Clinical, Commercial	Process Development / Optimization
22	bluebird bio	May-2017	Novartis	Lentiviral	Commercial	Product / Technology Licensing
32	Oxford BioMedica	Nov-2016	Orchard Therapeutics	Lentiviral	Clinical, Commercial	Manufacturing[69]
41	Kaneka Eurogentec	Jul-2016	Scancell Holdings	Plasmid DNA	Clinical	Manufacturing
59	Novasep	Jan-2016	Advanced Biotherapeutics Consulting	AAV	Lab, Clinical	Service Alliance[70]

[66] Information in this report (and specifically this table) has been identified from publicly available sources on a 'best-effort' basis. However, we realize that some of the data points may not be publicly available and, as such, may have been overlooked in our analysis. If you'd like to notify us of these gaps, please send an email to support@rootsanalysis.com

[67] There are few instances in the table wherein we could not directly find the information on the scale of manufacturing of the vectors. For such instances, the scale at which the vectors were expected to be produced has been estimated based on the phase of development (preclinical / clinical / commercial) of gene therapy / DNA vaccines that were the focus of the collaboration

[68] Source: https://www.prnewswire.com/news-releases/milliporesigma-signs-commercial-supply-agreement-with-bluebird-bio-for-viral-vector-manufacturing-300572800.html

[69] Source: http://www.oxfordbiomedica.co.uk/news-media/press-release/oxford-biomedica-announces-strategic-alliance-orchard-therapeutics

[70] Source: https://www.novasep.com/home/about-novasep/media-events/press-release/novasep-and-advanced-biotherapeutics-consulting-team-up-on-adeno-associated-virus-aav-vectors.html

S. No.	Company Name	Month-Year	Partner(s)	Vector Type	Scale of Manufacturing [67]	Nature of Collaboration
68	bluebird bio	Jun-2015	Kite Pharma	Lentiviral	NA	Product Development[71]
78	uniQure	Jan-2015	Treeway	AAV	Lab, Clinical	Product / Technology Licensing[72]

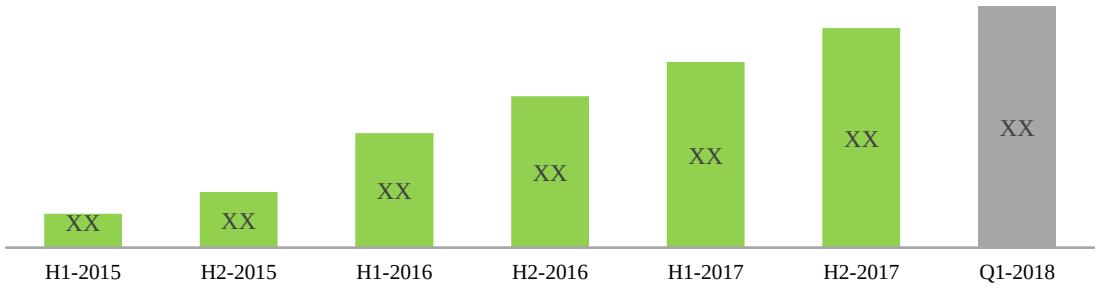
Note: The content represented in this table is illustrative, as the complete section is the proprietary property of Roots Analysis Pvt. Ltd. and protected by the company's copyright

Source: Roots Analysis

9.3.1. Analysis by Year of Partnership

Figure 9.1 represents the cumulative trend in partnerships and collaborations that have been inked between January 2015 and February 2018. With an aim to highlight the trend in the past three years, we have presented the collaborations on a half-yearly basis.

Figure 9.1 **Viral Vectors and Plasmid DNA Partnerships: Cumulative Year-wise Trend (2015–Q1 2018)**



Note 1: Data has been captured till February 2018
Note 2: The number in the boxes above the bar represent the number of collaborations that were established in that time period

Source: Roots Analysis

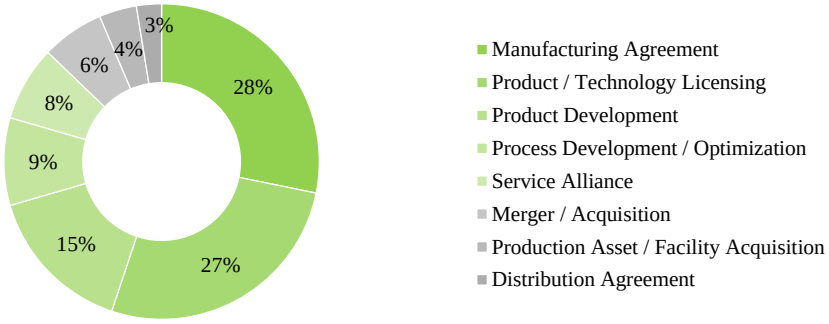
As can be seen from the figure, several companies are actively seeking opportunities to collaborate with other players in order to strengthen their manufacturing capabilities or utilize the manufacturing capabilities of other companies for production of viral vectors or plasmid DNA.

9.3.2. Analysis by Type of Partnership

Figure 9.2 represents the distribution of the collaborations on the basis of the type of partnership model.

[71] Source: http://investor.bluebirdbio.com/news-releases/news-release-details/kite-pharma-and-bluebird-bio-announce-strategic-collaboration
[72] Source: https://www.prnewswire.com/news-releases/treeway-announces-license-and-collaboration-agreement-with-uniqure-to-develop-a-gene-therapy-for-amyotrophic-lateral-sclerosis-als-288529301.html

Figure 9.2 Viral Vectors and Plasmid DNA Partnerships: Distribution by Type

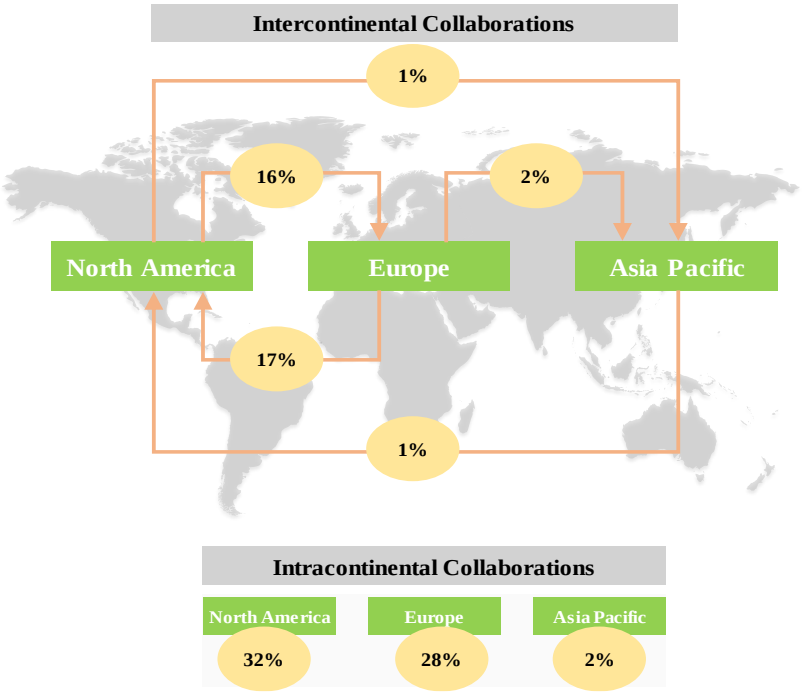


Note: Data has been captured till February 2018
Source: Roots Analysis

9.3.2.1. Intercontinental and Intracontinental Agreements

Figure 9.3 provides a quantitative geographical representation of the viral vector and plasmid DNA collaborations based on whether these agreements were signed between companies within a continent (intracontinental collaborations) or between companies whose headquarters are in different continents (intercontinental collaborations).

Figure 9.3 Viral Vectors and Plasmid DNA Partnerships: Regional Distribution by Intercontinental and Intracontinental Agreements



Note 1: Data has been captured till February 2018
Note 2: Agreements wherein more than two partners are involved have been counted multiple times
Note 3: The analysis does not include one instance for which the name of the collaborator is not disclosed

Source: Roots Analysis

9.4. OTHER COLLABORATIONS

In addition to the collaborations captured in table 9.2, wherein at least one partner has the capability to manufacture vectors, we came across few other agreements specific to vectors. These include agreements for research, discovery and development of vectors / novel vectors, development of vector component (such as vector capsids), manufacturing of vector based gene therapy / other genetically modified therapy. However, as the scope of our project was manufacturing of vectors, we have not included the aforementioned agreement types in the analysis presented in the earlier sections of this chapter. Table 9.2 provides a list of such agreements.

Table 9.2 **Viral Vectors and Plasmid DNA: List of Other Partnerships**[73]

S.No.	Month-Year	Partners	Purpose
1	Jan-2018	CGT Catapult and CombiGene	To develop manufacturing process for novel gene therapy product candidate[74]
5	Dec-2017	Biogen and University of Pennsylvania	To advance gene therapy and gene editing technologies[75]
10	Sep-2016	Voyager Therapeutics and California Institute of Technology	To license novel AAV capsids, intellectual property and related technology[76]
13	Jun-2015	GenVec And Washington University	To discover targeted adenovector-based therapeutics and vaccines[77]

Source: Roots Analysis

[73] The list may not be exhaustive
[74] Source: https://ct.catapult.org.uk/news-media/general-news/press-release-cgt-catapult-combigene
[75] Source: http://media.biogen.com/press-release/investor-relations/biogen-announces-collaboration-university-pennsylvania-multiple-gen
[76] Source: http://ir.voyagertherapeutics.com/phoenix.zhtml?c=254026&p=irol-newsArticle&ID=2202034
[77] Source: https://www.genvec.com/media/press-releases/detail/1790/genvec-and-washington-university-at-st-louis-form

CHAPTER 10

CONCLUSION

9.5. ORAL PROTEINS AND PEPTIDES MARKET: FUNDING AND INVESTMENT ANALYSIS

Over the years, advances in recombinant DNA technology and *ex vivo* synthesis of biomolecules have led to the development and (in some cases) approval of several protein / peptide-based therapeutics. However, owing to their inherent structural complexities and compromised stability (in *in vivo* conditions), proteins / peptides are primarily delivered via the subcutaneous or intravenous routes of administration. Recent strides in drug delivery solutions have enabled scientists to successfully explore and exploit alternative routes of drug delivery, such as transdermal, intranasal, pulmonary and oral, for protein / peptide-based therapeutics. Of these, the oral route of delivery is the most patient-friendly and, hence, several companies have invested their efforts in the development of biologics that can be delivered via the oral route.

The field of oral proteins and peptides has captured the interest of several drug developers, including both small to mid-sized players and large companies. While more than half of these pipeline candidates are in the discovery / preclinical stages, around 28% of drug candidates are presently in advanced stages of evaluation (phase II and above)

A key objective of this project was to determine the primary growth drivers and estimate the future size of the market. Based on parameters, such as target consumer segments, likely adoption rates and expected pricing, we have provided an informed estimate of the likely evolution of the market in the short to mid-term and long term, for the period 2018-2030.

9.6. VECTOR MANUFACTURING MARKET: PARTNERSHIPS AND COLLABORATIONS

Over the years, several gene-modified therapies have emerged as promising treatment options for various diseases (primarily those that till date have no definite cure), including different types of cancers, inherited disorders and some viral infections as well. Such therapies basically involve the introduction of a therapeutic DNA molecule (gene of interest), either directly (in a suitable vector) or indirectly (within genetically modified cells) into the patient’s body. The role of vectors, which are essentially various types of gene delivery vehicles, is critical to the discovery, development and production of these advanced therapeutic strategies.

One of the key objectives of this study was to evaluate the current opportunity and the future potential of the vector manufacturing market over the coming decade. Based on various parameters, such as the likely increase in the number of clinical studies, increase in the patient population, existing price variations among different vector types, and the anticipated success of commercial gene therapy products, the report provides an informed estimate of the likely evolution of the market in the short to mid-term and long term, for the period 2018-2030.

The research and analyses presented in these reports are backed by a deep understanding of insights gathered both from secondary and primary sources. This enabled us to solicit inputs on upcoming opportunities and challenges that were considered to develop estimates for a more inclusive growth.