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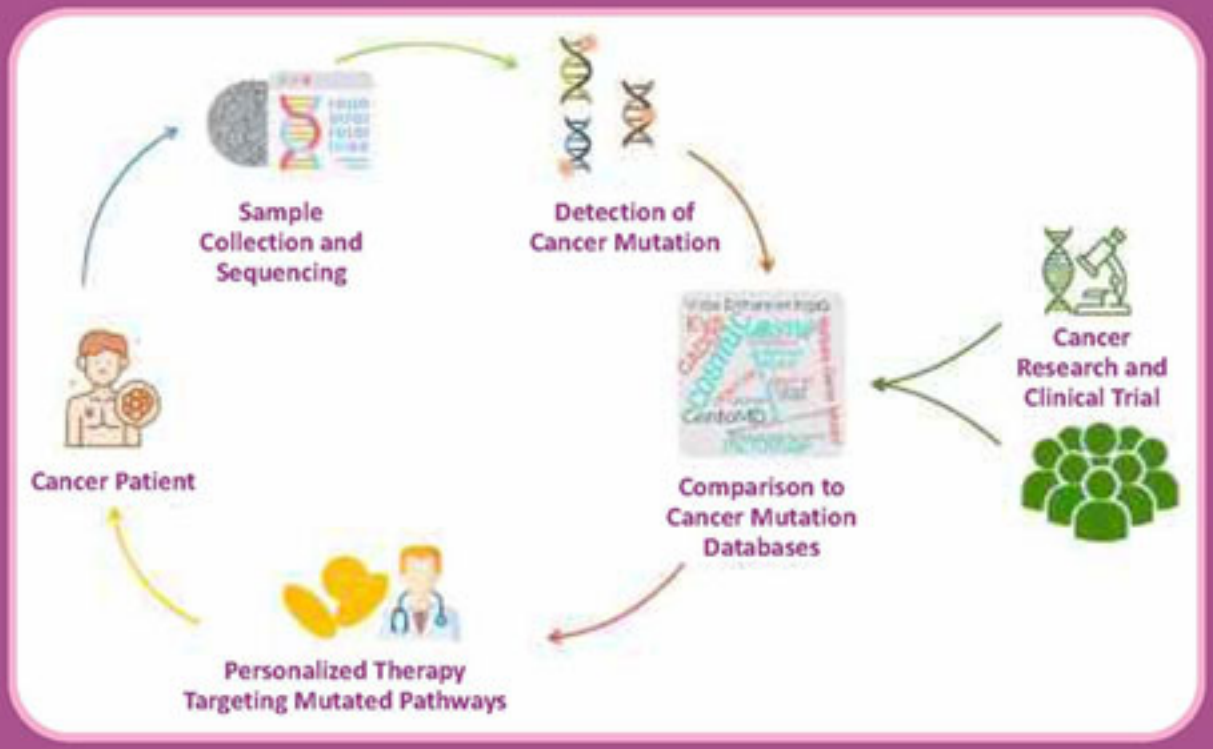

Peptide-based Pharmaceuticals: How to Strengthen Generics and Invigorate Innovation Ecosystems to Make 'Self-reliant India'

Genomics For All: Healthcare, Pharmaceuticals and Beyond

CRIPS Digest & NIPER News

National Institute of Pharmaceutical Education and Research (NIPER)
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The front page cover art is from the article by Dr. Gautam Das

EDITORIAL

The theme of the flagship programme of Azadi ka Amrit Mahotsav of the Department of Pharmaceuticals was "Story of Pharma @75: Future Opportunities". The celebration of 'NIPER Week' comprised of lectures and seminars by eminent academicians and leaders from the industry, and NIPER alumni, as well as exhibitions highlighting NIPERs' niche areas. In his inaugural address, the Hon'ble Union Minister of Chemicals and Fertilizers and Health & Family Welfare, Dr. Mansukhbhai Mandaviya Jee had exhorted the NIPER community to frame the pharmaceutical road map for the next twenty five years so that goals to be achieved at 100 years of independence are set. The second issue of revived CRIPS has two articles by the speakers of this august gathering.

The article by Dr. Ved Srivastava from Global Peptide Science Institute, USA, on peptide-based pharmaceuticals lays special emphasis on the status of the industry in India and some probable measures that the Indian industry can adopt to move from a generics-driven to innovation-based unit. The author has discussed five pillars in pharmaceutical sector which can support an Atmanirbhar India. The importance of peptide therapeutics and its status in India have been described. The advantages that India enjoys and how these can be mobilized to build an innovative peptide pipeline in India have been highlighted.

The article by Dr. Gautam Das and his group at miBiome Therapeutics, India, discusses the birth and growth of the area of genomics and the enormous data trove generated by the Human Genome Project. The evolution of next generation sequencing, with advances in sequencing machinery and the concomitant plunging cost of genome sequencing, have been described. The authors have highlighted the use of this continually developing technology in microbial-pathogen analysis in tuberculosis, the use of genome surveillance in containing the spread of SARS CoV-2, cancer therapeutics, etc.

On behalf of the editorial team, I invite you all to read this edition and provide your valuable feedback.

Prof. Ipsita Roy

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Peptide-based Pharmaceuticals: How to Strengthen Generics and Invigorate Innovation Ecosystems to Make 'Self-reliant India'

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This article summarizes the presentation at the Industrial Leadership Conclave: The role of pharmaceutical Industry in the development of India since independence organized by the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, which was sponsored by the Government of India, Department of Pharmaceuticals. The generic pharma industry of India, including peptides, is a major contributor to the world pharmaceutical economy and is considered the world's third largest by volume. Marching towards 100 years of independent India, it is time to reinvigorate innovation towards discovering novel drugs, self-reliant clinical development programs, and commercialization of innovative drugs. Peptide (smaller fragment of protein) therapeutics have continued to be an innovative strategy for the development of biopharmaceutical pipelines in the developed world, such as the USA. 'Peptide therapeutics' is still an untapped innovative area in India. This article covers (a) the status of biopharmaceuticals, including peptides in India, (b) paving the way from generic to innovative drugs and future directions for India, and (c) why peptide is an innovative approach to build a pipeline of patient-centric and first-in-class peptide-based drugs.

INTRODUCTION

The Status of Biopharmaceuticals in India

The increasing trend towards economics and biopharmaceutical assets in India is remarkable. Based on the published articles,[1-2] the generic pharma industry of India is considered the world's third-largest by volume. It has made a significant contribution to the world's pharmaceuticals economy. India is also the world's third-largest producer of recombinant Hepatitis B vaccine (a recombinant hepatitis B vaccine (GeneVac-B) manufactured by the Serum Institute of India, Pune. India supplies over 80% of the antiretroviral drugs used globally to combat AIDS.[1-3] The Indian biotechnology industry was valued at ~$64 billion in 2019 and is projected to reach~$150 billion by this decade.[2,3] India's domestic pharmaceutical industry includes a network of ~10,500 manufacturing units, ~3,000 drug companies,and ~5000 biotech companies.[3,4]

During the last decade, the Indian Government has taken initiatives and provided investment in building infrastructure for pharmaceutical development. For example, the Department of Pharmaceuticals of Government of India initiated a Production Linked Incentive (PLI) scheme to promote domestic manufacturing; and has taken many steps to reduce the cost of the drug production and cost of drugs to patients. Recently, the Government of India has set aside approximately $230 million for biotechnology R&D to set up nine biosafety level-3 (BSL-3) laboratories to advance biologics. State Governments such as Uttar Pradesh are also making a pitch for building a "bulk drug park" and a "medical device park" and investing in start-up India and make-in-India initiatives. All this funding support is to make India a hub for end-to-end drug manufacturing.[5]

Are these government initiatives and incentives good enough to make 'Atamnirbhar Bharat'? Is the R & D budget increase good enough to develop innovative drugs leading India to the 100th year of Independence? Given the innovations and initiatives, I believe India will surely boost and strengthen generic manufacturing for the short term but may not be sustainable for the long term to lead India to be self-reliant by its centennial.

Paving the Way from Generic to Innovative Drugs

The data from the United State's FDA and company members of the Pharmaceutical Research and Manufacturing Association (PRMA) reported

$83 billion for R & D expenses in 2019, including inflation cost, which is nine folds incremental increase from the 1980s and 1990s.[6] Drug approvals declined during the last four decades, despite steadily rising R&D spending over the preceding years. However, between 2015-2019 increased R&D spending resulted in increased new drug approval,[2] to which during this period, a large number of biologics were developed. The 'Atmanirbhar Bharat' resonates with innovative biotechnology and novel technologies assisting health care advancement.

Five vital disruptive technologies (Figure 1), in my opinion, could lead to self-reliant India - (1) Artificial Intelligence (AI) & Machine Learning (ML) both go hand in hand. For example, '*InVivo* AI', a Canadian startup developing a novel algorithm for drug discovery for central nervous system diseases; Peptilogic, Inc, a USA based start-up is designing computational algorithms for peptide-based drug design. (2) Precision Medicine: treating each patient as a unique individual, personalized medicine, neoantigen vaccine, e.g., The MEDi platform for rapid identification of tumor-specific antigens from the patient such as human leukocyte antigens. (3) Digital Therapeutics - non-pharmacological, tech-driven solutions as stand-alone or used in conjunction with medications and devices. For example, Dopavision, a German start-up, is making a smartphone-based digital therapeutic for myopia, eye treatment by activating dopamine. (4) Curative Therapeutics such as Cell Therapy. It is a paradigm shift in treating illnesses from managing diseases to curing diseases altogether. Mogrify Ltd., a British startup, is developing novel cell therapies for musculoskeletal, auto-immune, cancer immunotherapy and respiratory diseases. (5) Peptide Therapeutics is becoming an innovative strategy to develop novel drugs. Peptide Therapeutics is covered in detail in the subsequent sections.

PEPTIDE THERAPEUTICS OPPORTUNITIES

Why Peptide Therapeutics

Peptides are small fragments of proteins, a string of up to 40 amino acids. In recent years, peptides have received increased interest in pharmaceutical, food, cosmetics, and other fields. Therapeutics peptides are endogenous ligands that are efficacious and safe. Because of the safety and efficacy advantage, the attrition rate of biologics is very low. Out of 100 drugs entered into the clinical trials, 25% peptide-based make it to market, compared to only 13% small molecules because of their unpredictable safety profile.[7]

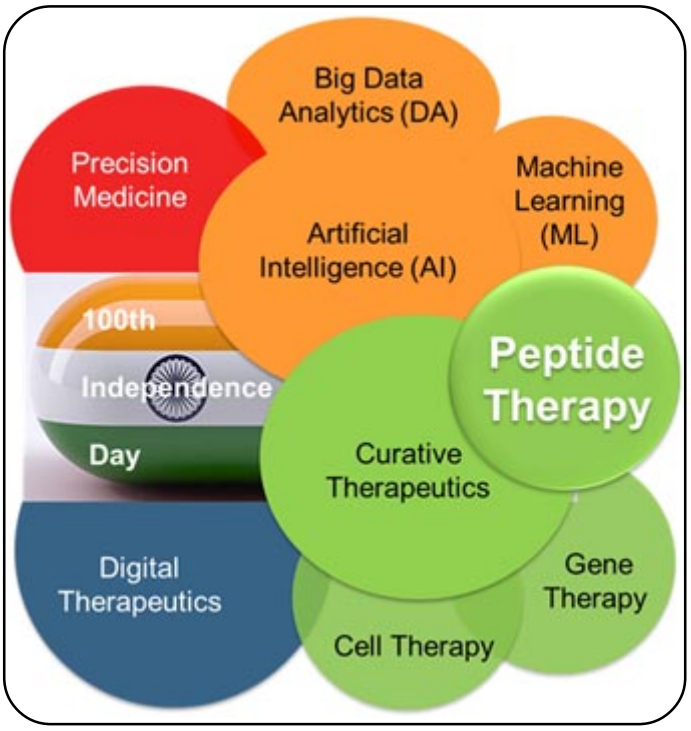

Figure 1- Future trends: disruptive technologies for self-reliant India.

During the last four decades, the discovery and development of peptide therapeutics have grown exponentially. More than one thousand peptide molecules are currently being studied for therapeutic indications in various disease areas, such as metabolic diseases, infectious diseases, cancer, and neurological disorders. More than 70 peptide drugs have been approved for marketing. More than 150 peptide molecules have entered into clinical trials for a wide variety of therapeutic indications, including metabolic, cardiovascular, oncology, and central nervous system diseases.[8] Most of the clinical and commercial successes of peptide therapeutics have been seen in metabolic diseases, and for peptide drugs acting on extracellular targets such as GPCRs, include the insulin analogs NovoLogs (insulin aspart). Some of the blockbuster drugs includes Humalogs (insulin lispro), Victozas (liraglutide), Byetta (exenatide), Luprons (leuprolide), Sandostatins (octreotide) and Forteo (teriparatide). The approved peptide-based drugs are listed in Table 1.

Peptide Discovery: The use of peptide therapeutics directed at intracellular targets such as transcription factors, kinases, and intracellular receptors, has been limited[9,10] due to challenges in investigating intracellular targets, target effectiveness /validation, and challenges in discovering and developing cell-penetrating peptides and understanding protein-protein interactions. Macrocyclic peptides have the ability to disrupt intracellular protein-protein interactions such targets are often considered 'undruggable.' The use of macrocyclic peptides opens new

Table 1. Approved Peptide Therapeutics (2020). The list is adopted from the book, Peptide Therapeutics: Strategy for Chemistry Manufacturing and Control (CMC)[10], Ed, Ved Srivastava, RSC, 2019.

Abeloparatide [2017]	Histrelin [1991]
ACTH [1-39] [1952]	Icatibant [2008]
ACTH [4-10] [2011], Russia	Lanreotide [2007]
Afamelanotide [2014]	Leuprolide [1984]
Alarelin, China	Linaclotide [2012]
Albuvitide [2018]	Liraglutide [2009]
Alloferon, Russia	Lisinopril [1987]
Angiotensin [1-7] [2017]	Lixisenatide [2013]
Atosiban [2000]	Lucinactant [2012]
Aviptadil [2000]	Lutathera [2018]
Bivalirudin [2000]	Macimorelin[2017]
Blenrep/Belantamabmafodotin [2020]	Mifamuritide [2009]
Bremelanotide [2019]	Nafarelin [1990]
Buserelin [1984]	Neogen, Russia
Calcitonin [human] [1986]	Nesiritide [2001]
Calcitonin [salmon] [1971]	NOV-002, Russia
Carbetocin [2001]	Octreotide [1988]
Carfilzomib [2012]	Ornithine vasopressin, Australia [1971]
Carperitide [1995]	Oxytocin [1962]
Cetrorelix [1999]	Pasireotide [2012]
Degarelix [2008]	Plecanatide [2017]
Desmopressin [1972]	Pramlintide [2005]
Detectnet/Gallium 68 PSMA-11 [2020]	Romiplostim [2008]
Dulaglutide [2014]	Scenesse [2019]
Elcatonin [1981]	Semaglutide [2017]
Eledoisin [1970s]	Setmelanotide/Imcivree [2020]
Enalapril [1985]	Somatostatin [1970s]
Enfuvirtide [2003]	Taltirelin [2000]
Eptifibatide [1998]	Teduglutide [2012]
Etelcalcetide [2016]	Teriparitide [2002]
Exenatide [2005]	Terlipressin [1978]
FAR-4043 [2010]	Tesamorelin [2010]
Felypressin [1970s]	Tetracosactide [1980]
Ganirelix [1999]	Thymodepressi, Russia
Glatiramer [1996]	Thymopentin [1985]
Glucagon [1989]	Thymosin-α_1 [2009]
GMDP, Russia	Triptorelin acetate [1986]
Golotimod, Russia	Triptorelin pamoate [2009]
Gonadorelin acetate [1989]	Vapreotide [2005]
Goserelin [1987]	Vasopressin [1962]

opportunities to address a range of human diseases such as cancer and cardiovascular disease.[11] While much progress has been made in developing peptide therapeutics over the past several decades, we still need to understand better (1) the pharmaceutical properties required for drug-like peptides, (2) the correlation of nonclinical PK/PD that can translate to humans, and (3) appropriate peptide delivery technologies.

Chemistry, Manufacturing, and Controls (CMC): Significant progress has been made toward the cost-effective manufacturing of the Drug Substance (DP or API) and Drug Products (DP).[12] There is a lack of clarity in Chemistry, Manufacturing and Controls (CMC) strategy, encompassing clinical development to commercialization. This could be one of the potential barriers to the development of novel peptide drugs. CMC can often become a rate-limiting step for peptide-based drugs owing to a deficiency in knowledge and a lack of formal policy or CMC guidelines. Despite several successes, specific regulatory challenges are often related to managing quality standards. These challenges are partly due to a lack of official regulatory guidelines for peptide drugs, as there are no current FDA or International Conference on Harmonization (ICH) guidelines that address the quality of pharmaceutical peptide products. Regulators frequently use a risk-based assessment on a case-by-case basis when reviewing NDAs (New Drug Applications) or ANDAs (Abbreviated New Drug Applications). The system for regulating medicines in Europe is unique in the world. It is based on a closely coordinated regulatory network of competent national authorities in the Member States of the European Economic Area (MS-EEA) working together with the European Medicines

Agency (EMA) and the European Commission.

Delivery Routes for Peptides Drug: Peptides are being delivered via invasive parenteral route; however, several non-invasive delivery routes such as nasal, buccal, transdermal, and pulmonary have been investigated, particularly for chronically administered drugs.[10,11,13]

Peptide drug molecules are generally not delivered orally because of their poor aqueous solubility and poor membrane permeability in the gastro-intestinal (GI) tract, leading to unacceptably low oral bioavailability. The oral route is a better option because of its patient-friendly delivery and increases the drug's therapeutic value. However, a few peptide drugs are approved for oral delivery, but they are intended for the GI restricted therapeutic targets, e.g., Vancomycin (Vancocin®), cyclic peptide, modified amino acids, 1449 Da, as gelatin capsule for Staphylococcus enterocolitis and Clostridium difficile-Associated Disease (CDAD), Staphylococcus enterocolitis. Fidaxomycin (Dificid®), macrolide compound, 1058 Da, as tablet for CDAD, Linaclotide (Linzess®), cyclic peptide, 1525 Da, as gelatin capsule for CIC and IBS-C. A few peptide drugs targeted for systemic delivery are currently marketed, e.g., Desmopressin (DDAVP®),1128 Da, as a tablet for central diabetes insipidus, nocturnal enuresis; Cyclosporine (Neoral®), 1202 Da, as self-emulsifying system, for organ transplant rejection and Taltirelin hydrate (Ceredist®) 477 Da, as orally dissolving tablets, for spinocerebellar degeneration. Several biotech and large pharma companies are investing in developing the technologies for oral delivery of peptides, and most recently Semaglutide, (RYBELSUS®), 4113 Da as tablets was approved by FDA for lowered blood sugar and body weight.

Implantable technologies can facilitate delivery of a controlled concentration of drug to a patient by controlling the rate of drug release.[13] There are three critical components of implantable drug delivery systems: (1) a highly potent drug payload (peptides), (2) a formulation providing long-term thermal stability to the payload, and (3) precisely controlled drug release mechanisms via zero-order kinetic release, or pulsatile. Significant progress has been made toward developing various implantable technologies to deliver drugs via intracranial, intrathecal, or intravaginal routes. However, the most promising developments have been in intraocular and subcutaneous implants. Some of these technologies have gained FDA approval in recent years. Research and development in this area continue to focus on the need for both implantable devices and in situ-forming implant technologies. These implantable technologies may contain therapeutic agents in nanomaterial formulations of non-bioabsorbable and biodegradable polymers.

PEPTIDE THERAPEUTICS IN INDIA

Why Peptide Therapeutics in India ?

Based on the 2017 Indian market report,[14] sales of peptides manufactured in India, including Heparin were approximately $380 million and expected to reach over $800 million by 2022. A Compound Annual Growth Rate (CAGR) of 15.0% was registered. India manufactures more than 30% of the generic peptide-based drugs, including Active Pharmaceutical Ingredients (API). Some of the peptide-based generic drugs include Insulin, Liraglutide, and Exenatide for Diabetes; Eptifibatide for infectious diseases; Leuprolide and Octreotide for prostate cancer; Bivalirudin and Glatiramer for cardiovascular (CV); and Forte and calcitonin for Osteoporosis; and Oxytocin for Uterine contraction.[15] India is the largest supplier of Oxytocin and Bivalirudin and is expected to register the highest- CAGR during this decade.

The key players in India for peptide-based drugs include Aurobindo, Biocon, Cadila, Cipla, Dr. Reddy, Natco Pharma, Neuland, SunPharma, USV, Wockhardt and Zydus. Hemmo Pharmaceuticals, the largest producer of oxytocin, recently acquired by Piramal Pharma Ltd. Neuland Ltd, is an expert in manufacturing of starting-material for peptide drugs and is believed to be one of the largest suppliers of Pseudoproline, an intermediate for complex peptide manufacturing. ISSAR Pharma (now Cadila), to my knowledge, was India's first-ever company to conduct Phase-1 clinical trials and launch the first-ever indigenous peptide drug, Melgain for the treatment of Vitiligo, a skin disorder that causes the skin to lose its color, in 2004. ISSAR has patented novel formulation for a few other drugs, e.g., Xylentra® contains an active drug substance called Genopep®, a 23 amino acid peptide, and Novoskin (10 aa), a Basic Fibroblast Growth Factor (BFGF) as topical formulation.[16,17]

The patent expiry of blockbuster prescription drugs will provide additional growth to India. More than 70 peptide-based drugs are on the market worldwide, and more prescription-based peptide drugs may soon become generic due to potential patent expiry, such as Byetta or Victoza.

HOW TO BUILD INNOVATIVE PEPTIDE PIPELINES IN INDIA

India has the capabilities, capacity, infrastructure, know-how, and technical expertise to strengthen and expand the generic peptide drugs and API

manufacturing to enhance its revenue. With 25% of the revenue earned and supplemented by the Government initiative, now it is the time to leverage these assets to develop India's own innovative peptide drugs and build a self-reliant India by its centennial.

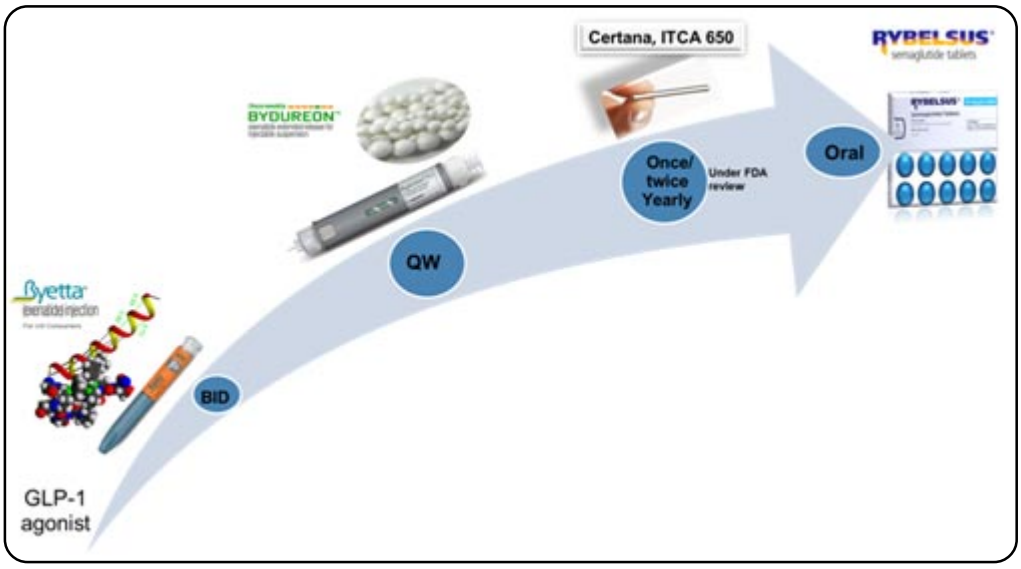

Figure 3. GLP-1 agonists form twice-a-day (BID) injection to oral delivery via innovative formulation. The logo images of the drugs taken from their website.

Strategy and Technology

One of the approaches to build innovative peptide pipelines in India could be as illustrated in Figure 2. The horizontal scale represents the Risk and Revenue, and the vertical scale represents Reward and Self-reliant. There could be three phases to progression path for a self-reliant India (a) during the next five years, India continues to develop cost-effective production, increase larger-scale production of Generic peptides by volume,expand generic peptides or API products beyond the current pipeline. It is a low risk and rewarding effort, (b) in the second phase of five years, develop novel formulations of the currently marketed generic peptide drugs for alternative delivery such as nanoparticle-based delivery or oral delivery. For example, formulation-based new innovative products of Exenatide, as life cycle management, illustrated in Figure 3, and (c) in a third phase of five years, India should take a leadership role in innovative, first-in-class commercialization peptide-based drugs using disruptive technologies. This ultimate phase would lead to a high risk, high reward, and high revenue value. These three phases mentioned above could be executed sequentially or as staggered to ram up towards self-reliant India by centennial. Of course, it requires aggressive efforts and time to make a self-reliant India in the prescription of peptide-based medicine.

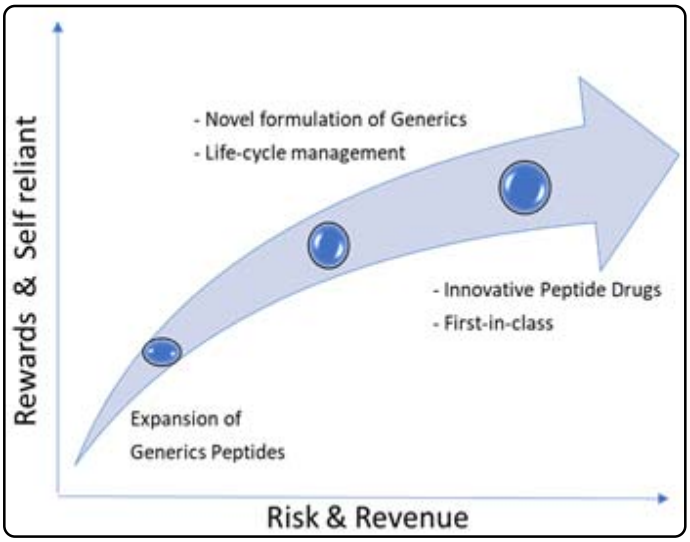

Figure 2. Progression path of peptide drugs development towards revenue and self-reliant India.

Manufacturing Advantage

The number of peptide drugs entering the clinical trial has increased exponentially during the last four decades. The market size of peptide drugs, especially APIs, has increased significantly. A few decades ago, marketed peptide drugs were smaller in length, mostly 10 amino acid sequences, but they are more than 30 or 40 amino acids in length in the current decade. We now have state-of-the-art techniques for the pharmaceutical characterization of larger peptide sequences and better manufacturing technology on kilogram scales. Based on my personal experience in the current decade, the cost for a 30 to 40 amino acid peptide sequence on 10 kilogram/batch is USD ~$600/gram compared to $2,000/gram in the 1990s.

Technical Expertise

In addition to technologies, building innovative peptide pipelines in India requires a transformation of scientific talent from peptide manufacturing toward sinnovative peptide drug discovery to make India more self-reliant.

Based on the membership of the Indian Peptide Society,[18] India has more than 300 researchers engaged in peptide science in academic institutions such as AIIMS, NIPER, ICGB, JNU, IIT, IISc, and Biotech organizations. We need to reinvigorate them by advanced training or motivate them towards application-based and patient-centric research and innovation of drug development. In my opinion, Academia prefers publications; individuals prefer discoveries but are

bound by the expectations of long-standing elitism created by the Academic industry. Academic scientists need to transform themself from a publication mindset to a patent mindset. Patents filed in Biotech subject matter by Indian residents' biotech scientists are only 30%, whereas non-resident Indian biotech scientists are 70%.[19]

Transform Talent

India's NIPER institutions have prepared their students to be a part of the state-of-the-art technologies in peptides, computer-aided drug design, and pharmaceutical sciences. The institution has transferred or licensed its technologies to drug manufacturers and secured several patents. The students' alumni have been recognized with prestigious Awards such as Thomson Reuters Innovation Awards.

In 2019, approximately 590 students graduated from all the seven NIPER Institutions with a major in Pharmaceutical Sciences.[20] Today it is fair to ask what percentage of these 2019 graduates from all the NIPERs are continuing or furthering their career towards innovative drugs discovery. If more than 50% of them retain their passion for drug discovery and development within India's Pharmaceutical Industry, it is a reasonable talent 'brain grain'. Institutions need to encourage students further the importance of professional development with cutting-edge science and to invest in self-management, communication (written and verbal), and leadership skills. It is the right time for all the NIPER students to make a global impact and stay the course every day when they come to the campus. A similar concept could be applied to other educational institutions within India.

Filling the Gap in Biotechnology

While India fosters the talents of students and cultivate their skills to further contribute to the scientific community around the world, it is time to expand the innovation scope, leverage veteran expertise and experience to fill the gap of innovative technologies or/and leadership within India. Like any other information technology (IT) or telecommunication sector, it is time for the biotechnology sector to aggressively engage Indian scientific experts settled abroad as Scientific Advisors.

Knowledgebase Resources

There is an abundance of information on peptide-based therapeutics and understanding the technology that will help to build the necessary basic knowledge to become innovative. Most recent books on peptide-based therapeutics[9-13] are excellent resources for starting or advancing peptide therapeutics in India. Collectively, these books provide (a)holistic story from molecules to medicine, combining the themes of design, synthesis, biomarkers, and clinical applications of peptide-based therapeutics, (b) regulatory process both in America and Europe, and guidelines including immunogenicity evaluation strategy, (c) the manufacturing process, and quality control strategy, novel techniques for characterization of peptide impurities and stability testing, and (d) peptide formulation, nanoparticles, peptide drug delivery (including a major emphasis on implantable drugs delivery for chronic diseases).

CONCLUSION

The Atmanirbhar Bharat resonates with advancing bio-innovation in biotechnology/peptides therapeutics as one of the avenues. The generic pharma industry, including peptides in India, is considered as the world's third largest by volume. The transformation from Generic to Innovative drugs in India includes adopting new technologies, fostering pharmaceutical talents, and filling the gap in the technology by levering peptide expertise beyond India. The peptide-based innovation could start with the innovative formulation of the current generic peptide drug to a patient-centric and patient-friendly peptide drug delivery. For example, as illustrated in Figure 3, Exendin-4 (Byetta™), a GLP-1 agonist was developed as a twice-a-day injection, it was followed by the development of Bydureon™ (once-a-week injection), and then the Certain™, (ITCA-650) (once or twice-a-year implant), still under FDA review. In 2021, Rybelsus,™ GLP-1 analogs, was approved asan oral tablet(once-a-day). These are great examples of life cycle management of a peptide-based molecule for Type 2 Diabetes treatment. The Government of India's initiatives and investments to strengthen cost-effective generics and biosimilars are admirable[6] and provide encouragement to innovative drugs and enhancing manufacturing of APIs.

REFERENCES

1. https://finmedium.com/2021/06/biotechnology-industry-in-india-size/, 2021
2. http://www.pharmabiz.com, April 2021
3. https://www.ibef.org/industry/science-and-technology.aspx, June 2021
4. www.cbo.gov/publication/57025#data
5. https://www.ddw-online.com/drug-discovery-and-india-the-future-looks-bright-10649-202104, Vol 22, 2021
6. https://www.cbo.gov/publication/57126, April 2021

7. KMR Group, Pharmaceutical Benchmarking Forum (PBF), 2012
8. Nester J.J Jr, Curr. Med. Chem., 2009,16, 4399
9. 'Peptide 2015', https://www.americanpeptidesociety.org/assets/pdf-files/proceedings/24.pdf
10. Comprehensive Medicinal Chemistry III - Volume 7: Biologics Medicine, www.elsevier.com
11. Peptide-based Drug Discovery: Challenges and New Therapeutics, https://pubs.rsc.org/en/content/ebook/978-1-78262-732-6)
12. Peptide Therapeutics: Strategy for Chemistry Manufacturing and Control,https://pubs.rsc.org/en/content/ebook/978-1-78801-433-5)
13. Implantable Technologies: Peptides and Small Molecules Drug Delivery, https://pubs.rsc.org/en/content/ebook/978-1-83916-222-0)
14. https://www.researchandmarkets.com/reports/4380160/indian-peptides-and-heparin-market-by-type, 2017
15. https://www.flagshipbiotech.com/peptides-products-manufacturers-in-india.html
16. https://xylentra.com
17. https://novoskin.in
18. http://indianpeptidesociety.com/otp
19. Vedachalam N., "India's Innovation Ecosystem: Mapping the Trends," ORF Issue Brief No. 442,page 12, February 2021. https://www.orfonline.org/research/indias-innovation-ecosystem mapping-the-trends
20. https://pharmaceuticals.gov.in/nipers, Annual Report 2019-2020

Genomics For All: Healthcare, Pharmaceuticals and Beyond

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Introduction

Genomics is the study of an organism's genome, its structure, its function, and its interaction with the surrounding environment. A succession of nucleic acids in the form of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) stores the information required for an organism's structure and its function. To understand the content of the genetic information, it needs to be sequenced. Increased accuracy, increased throughput combined with plummeting cost of DNA sequencing has made genomics an essential tool for probing biological systems. Genomics is routinely used in areas of genotyping, epigenetics, gene editing, structural genomics, metagenomics, epidemiology, and functional genomics. In the field of human genetics and medicine alone the genomics revolution has helped us in answering questions such as what makes us unique, how are we different than other people or living beings around us, tracing our ancestry, and sketching the path of human evolution, genetic bases of a number of diseases like cancer and genome driven drug discovery. The vast trove of data generated using genomics has helped in its application to fields such as medicine and public health, forensics, agriculture, bioengineering, diagnostics, and consumer products.[1] Unquestionably genomics was the most essential tool for epidemiological surveillance during the SARS-CoV-2 pandemic.

In this chapter, we take a sneak peek at the evolution of genomics and then discuss its application in infectious disease, in the current pandemic, in oncology and its application in metagenomics.

BOX / Glossary

What is DNA?

Deoxyribonucleic acid (DNA) is a contiguous sequence of four nucleotides - Adenine (A), cytosine (C), guanine (G), and thymine (T). Two such polynucleotide chains wrap around each other to form a double-helical DNA molecule. In nearly all organisms DNA carries genetic information.

What is a gene?

Gene is a segment of DNA coding for the synthesis of either RNA or a protein. The information in the gene is copied into RNA via a process called transcription. The RNA could be functional by itself or could serve as a template for protein synthesis. Proteins form the body structure of an organism, carry out the metabolic reaction and coordinate all the functions.

What is Genome?

Genome is the entire set of an organism's DNA/RNA. The genome contains all the information required for the organism to function. The human genome is made up of approximately 3 billion base pairs and contains about 30000 genes.

What is DNA Sequencing?

DNA sequencing determines the order of occurrence of nucleotide bases (C,G,T and A) in a segment of DNA. Reading the sequence of bases helps determine the source of the DNA segment, whether the DNA segment codes for a gene or RNA or regulatory elements, and whether changes in DNA are disease-associated.

What is Metagenomics?

Metagenomics is the study of a collection of microbes collected from their natural habitat. As the name suggests (meta + genomics), it involves sequencing the genetic material (DNA or RNA) of many organisms together.

From base-pair to bedside - How did we get here?

The word "genomics" is more modern than the field of genomics itself.[2] The field of genomics was initiated with the discovery of genes as the inheritable material by Johannsen at the beginning of the 20th century.[3] It took several decades to recognize DNA as the basic heritable material and unravel its three-dimensional structure.[4] The later discovery by Watson and Crick in 1953 and sequencing of the first polypeptide chain by Sanger paved the way

for DNA sequencing. The approach of protein sequencing by Sanger included random fragmentation, reading the content of the fragment, overlapping the read fragments to yield a complete polypeptide chain. The basics of protein sequencing were retained for the first RNA sequencing and ultimately for the first DNA to be sequenced.[5] Sanger used the "plus and minus" method of DNA sequencing to sequence the first DNA genome. The same year Sanger introduced the chain termination method of DNA sequencing which became the most widely used DNA sequencing method for almost four decades. With an accuracy of 99.99%, chain termination or Sanger sequencing is still considered the gold standard.

Sanger sequencing

The Sanger method relies on spiking a small amount of fluorescently labeled dideoxynucleotide triphosphates (ddNTP - ddATP, ddTTP, ddCTP, and ddGTP) with normal dNTPs to the DNA synthesis mixture.[6] Each base is labeled with a different colored fluorescent dye. The general convention being A is labeled with green, T by red, G by black, and C by a blue dye. Unlike dNTP, ddNTPs lack the capability to form the phosphodiester bond with the next nucleotide leading to chain termination. During the reaction, DNA polymerase incorporates ddNTPs at random resulting in millions of copies of DNA fragments terminated at random lengths. The DNA fragments are sorted by size using gel electrophoresis. In the automated machine a laser excites the fluorescent dye and the computer detects the identity of each terminal ddNTP. The fluorescent intensity is translated into peaks along the length of the DNA being sequenced.

The continuous improvement and automation of Sanger sequencing aided in sequencing from genes to genomes of microorganisms and eventually some multicellular organisms. Sequencing created a massive amount and a need to store and share the data. GenBank was established in 1982 with just about half a million bases but grew exponentially to over 40 million bases by 1990.

The Human Genome Project

The new era of genomics was ushered by the Human Genome Project (HGP) - an exploration of what lies within us. It started in the year 1990 and took a bit more than one decade to publish the first draft of the human genome. In addition to providing the first glimpses into complete human genome, HGP accelerated advances in sequencing technologies, newer companies entered the sequencing market and adoption of genomics took place at an unanticipated scale. At the start of HGP Applied Biosystems undisputatedly ruled the market. In contrast at the conclusion of HGP 454, Solexa, Illumina, Agencourt, Complete Genomics, Applied Biosystems and Ion Torrent were offering sequencing technologies with higher throughput and reduced cost.

Next Generation Sequencing

The first alternative to Sanger sequencing came in the form of pyrosenquecing produced by Pyrosequencing AB and marketed by 454 Roche Life Sciences. Pyrosequencing relies on detection of light when there is pyrophosphate release. To the reaction mixture of single stranded template DNA, DNA polymerase, ATP sulfurylase and firefly luciferease, one of the four nucleotides is added. The intensity of light emitted on nucleotide incorporation is measured to determine how many nucleotides have been incorporated indicating the presence of complementary nucleotides of template strand. The nucleotide mixture is removed and a new nucleotide is added to the mixture and the process is repeated. Pyroseqeuncing was the first next generation sequencer and could produce 400-500 bp long reads with 99% accuracy. The reason why pyrosequencing revolutionized the sequencing space was its throughput and reduced cost. Pyrosequencing could produce up to 25 million base pairs in a single run and it costs one-sixth compared to Sanger sequencing.[5]

Although a number of sequencers were launched after pyrosequecing, the next leap in genomics came with the introduction of Illumina sequencers. Illumina commercialized the 'sequencing by synthesis' technology that relied on reversible dye-termination chemistry. Similar to Sanger sequencing, a fluorescently labelled reversible terminator dNTP is imaged on incorporation. The terminator is then cleaved and the process is repeated with next reversible dye-terminators. In 2014, Illumina had captured 70% of the DNA sequencers market and more than 90% of sequencing data being generated was from Illumina machines. Illumina also helped realize the goal of $1000 per genome in 2017.

Short read technologies like Illumina and 454 had a great impact on the field of genomics but they have their drawbacks. By their very nature, short read technologies cannot characterize repetitive genomic regions, extreme GC content, structural variants or genomes with multiple homologous elements. Even advanced bioinformatics algorithms can help only to a certain level. Repeats create unresolvable loops in the assembly graph that leads to discontinuous genome. Short read technologies use PCR amplification during library preparation step.[7] PCR amplification are inherently biased against regions containing extreme GC content. These regions ultimately lead to assembly as incomplete smaller

fragments.

Third Generation sequencing technologies

The gap left by second generation sequencing technology has been partially overcome by third generation long-read sequencing.[8] In 2011 Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) started providing long read sequencing. ONT has produced reads upto 2 million base pairs in length, though 10-30 kB reads are more common.[9] Long reads combined with absence of PCR amplification circumvent the problem of fragmented genomes. However, the high error rates of long-read sequencing set a challenge for accurate genomic analyses.[10]

To overcome the limitations of short and long read technologies and using both to their utmost potential hybrid genome assemblies are being used. Long reads can be used to resolve ambiguous loops in the assembly graph created by highly accurate short read technologies. Long reads can also be used to sequence extreme GC regions leading to less fragmented genomes. Hybrid assembly has been used to sequence human, plants and other non-human model organisms.[5]

An integral part of genomics is analysing the huge amount of data that is generated from the sequencers.

Genomics in Infectious Diseases

While considering infectious diseases, Tuberculosis (TB), caused by the Mycobacterium tuberculosis complex (MTBC), is among the leading causes of mortality, i.e. about 2 million people each year globally.[11] In 2019, an estimated 10 million fell ill with tuberculosis globally. Around 1.5 million die of tuberculosis every year globally. Acting upon its survival instincts, similar to other life forms, MTB strategically adapts to develop into MDR-TB, primarily attributed to inadequate diagnostics, inconsistent/ partial treatment and antibiotic drug abuse.[12] MDR-TB is typically characterised by development of resistance in MTB against at least isoniazid and rifampicin, the two most powerful anti-TB drugs.[13] For diagnosis, Sputum-smear microscopy is a common choice of test, however it does not detect extra-pulmonary or smear-negative TB, let alone the screening for various MDR-TB strains.

Different methods for drug resistance profiling in tuberculosis include:

- Phenotypic (DST): First line drugs: R,H,E,Z; Second line drugs: S, Lfx,Mfx, Km,Cm, Am; Other drugs: Lzd, Cfz, Bdq, Dlm, PAS etc
- Growth based drug susceptibility testing
- Rapid drug resistance testing (Genotypic: DRT)
- Nucleic acid amplification based (NAAT): Cartridge (GeneXpert) and Chip (TruNAAT)
- Line probe assay (LPA): First line (R:Rifampicin, H:Isoniazid); Second line (Lfx, Mfx, : Fluroquinolones, Km, Cm, Am: Aminoglycosides)

Genomic techniques play a dynamic role in the control of infectious diseases such as MTB by providing a rapid yet accurate and comprehensive microbial-pathogen analysis. The options include whole-genome sequencing (WGS) and targeted NGS (tNGS). While, WGS in clinical settings would require initial TB culture step so as to generate sufficient bacterial load, tNGS has emerged as a feasible option for faster, comprehensive, and importantly direct sequencing form patient samples.[11]

Application of Next-Generation Sequencing in tuberculosis drug resistance management carried out at miBiome Therapeutics LLP include-

- Cultureless method, faster DR profiling from sputum, Bronchoalveolar (BAL), Cerebrospinal fluid (CSF), ascitic fluid, biopsy or any clinical sample
- Querying Novel Indian isolate specific mutations
- Amplicon based sequencing including targets that distinguish non-MTB from MTB in addition to targets for drug resistance markers
- MDR, pre-XDR and XDR known biomarker panels and novel biomarker panels designed in-house

Therefore, the early detection of Pre-XDR/XDR-TB could guide clinicians in the appropriate adjustment of MDR-TB treatment regimen with effective drugs to prevent treatment failure

How genome surveillance is shaping the SARS-CoV-2 pandemic:

The application of Next-Generation Sequencing during the SARS-CoV-2 pandemic has been the classic example of the advantages of NGS which helped guide the public health response to a pandemic in near-real time. This is inclusive of first distinction and identification of the novel corona virus, screening and prediction of mutations (Genotyping variant analysis), and furthermore, developing screening test for infected population. NGS has played a major role during the pandemic in diagnosis which eventually is aiding in vaccine and drug target selection.

Whole genome sequencing (WGS) of SARS Cov2 is being used for genome surveillance. Although being an excellent tool, application of WGS for public health programs can be very expensive. To overcome such challenges, our team at miBiome Therapeutic LLP have come up with a unique solution for SARS-CoV-2 surveillance. This is inclusive but not limited to the following-

- Using evolutionarily unique regions and targeted

amplicon sequencing with in-house-designed unique/specific primer sets which target only SARS-Cov-2.

- Being targeted sequencing (only 14% of the genome) and not WGS, our method is considerably lower in cost than WGS.
- Interestingly, the required predominant information (>75%) to track current SARS Cov-2 variants is retained in the evolutionarily unique regions, thus not compromising on the genome information required for tracking.
- The lesser data output needed per sample increases the power of multiplexing and endows performing genome surveillance at a cheaper cost.
- To aid in mass screening, 7680 samples can be sequenced in one run using Juno.

Being a part of service providers in the field of NGS, in addition to our above mentioned solution in SARS-CoV-2 and MTb, the other services are presented in the Figure 1.

Genomics in Cancer

Cancer accounted for approximately 10 million deaths in 2021.[14] Genomic aberration such as point mutations, insertions and deletions, variation in copy number are hallmarks of many cancers. NGS can be used to detect these mutations in cancerous genes aiding in diagnosis, prognosis and advance personalized treatment.[15] Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES) and targeted gene panels are being used both in cancer research and clinical settings. Although WGS gives a broader perspective, in clinical setting it might become time and cost prohibitive. WES sequences only the coding region which is a bit more than 2% of the total human genome. Hence, WES in the method of choice when probing mutations in genes coding for proteins. Another time and cost effective method is targeted gene panels. As the name suggests targeted gene panels focusses on a selection of genes specific to a type of cancer. A number of targeted gene panels marketed by Illumina and LifeScience Technologies has seen a rapid uptake in the market in recent years.

As the NGS approach is novel, its use in diagnosis has not been fully realized neither the guidelines for its application in clinical setting has been formalized. In a wide variety of cancers. NGS has started to emerge as a diagnostic tool and for many more NGS-based diagnostic tools are in development. BRAC gene test, which tests for mutation in BRAC1 and BRAC2 genes, are recommended for early onset breast cancer patients or patients with family history of breast cancer. Sequencing of BRAC1 and BRAC2 genes using the Sanger method requires longer times and higher costs as these genes are made up of more than 20 exons each.[16] In addition to reducing cost, NGS helps in reducing the turnaround time of analysis and hence reducing the clinical reporting time. More number of early onset associated genes and high risk genes are being discovered and sequencing panels targeting these genes have been developed. Lin et al., developed a panel of 68 high risk breast cancer genes.

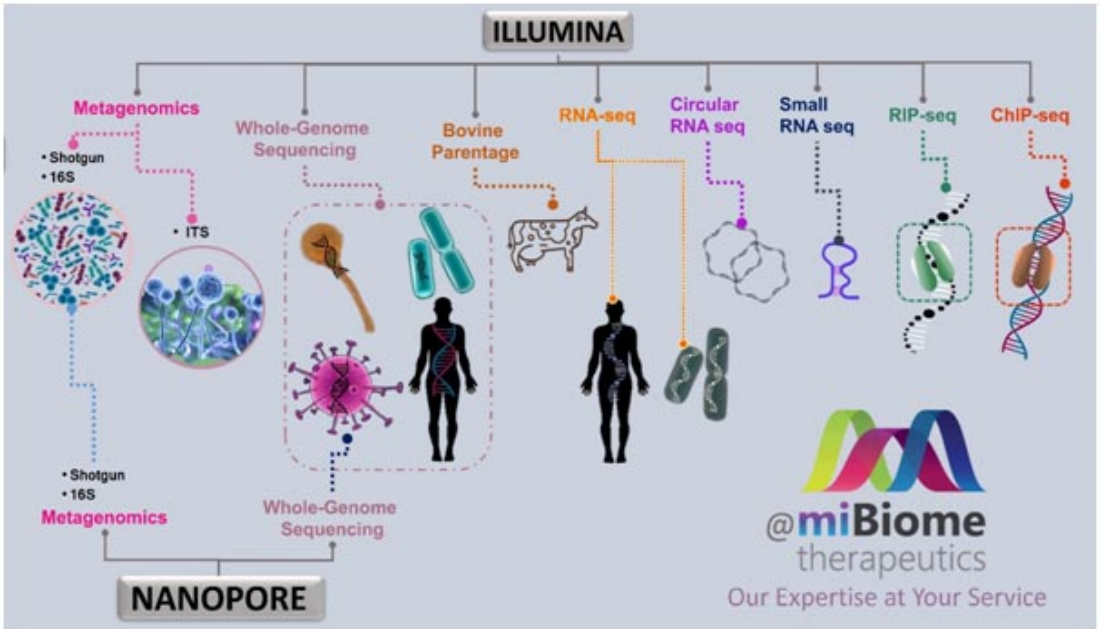

Figure 1: Services at miBiome Therapeutics: miBiome therapeutics is a leadingservice provider for NGS - based metagenomics, Whole genome sequencing, bovine parentage determination, RNA-seq, circular RNA-seq, small RNA-seq, RIP RNA-seq and ChIP-seq. Available with Illumina and Nanopore platforms.

NGS is being used for diagnosis of other cancers such as Lung Cancer, Colorectal Cancer and is being used in selection of therapy. For example, EGFR is targeted by several drugs in colorectal cancer. Relatively a smaller number of CRC patients can benefit from treatments targeting EGFR. Hence, prediction of treatment efficacy, reduction of side effects and cost can be achieved through detection of mutations in KRAS gene.

Other NGS based techniques like RNA-seq is being used to detect gene-fusion events, alternatively spliced transcripts, changes in expression of gene in cancer cells with respect to control or normal

cells. Few targeted gene panels to detect gene fusion events are based on RNA-seq in hematologic tumors. NGS are also being used to understand epigenetic changes, detect microRNAs and other small RNAs.

Genomics has made personalized treatment for cancer patients a realty (Figure 2). From treatment that was organ-centric to analysing the molecular details of cancer for treatment choice, cancer genomics is changing how we look at cancer diagnostics and treatment.[17]

Metagenomics in human health and Disease

Microbes are ubiquitous in nature and their specific composition is reflected in their ecosystem functionality.[18,19] The human body, as a host, has co-evolved with its microbiome, where some commensals evolved to be symbionts and some as pathobionts.[20] Additionally, microbiome architecture varies within different hosts and is primarily impacted by the physical environment.[21] In turn, various biochemical and bio-physical activities expressed by the microbial community, impacts the host metabolic functioning. Thereby understanding these microbes as influencing factors in host heath is assimilating attention. However, the intricacy of the microbial consortia/network makes its identification, profile prediction and mechanism of interaction challenging.[22] Since the traditional approaches of cultivation-based techniques provides a spurious overview on the microbiota, the culture-independent methods, based on PCR have proven to be an effective alternative and paved way for metagenomics. Metagenomic sequencing options have enabled unbiased systemic characterization of microbial communities, furthering therapeutic strategies in near future.[23]

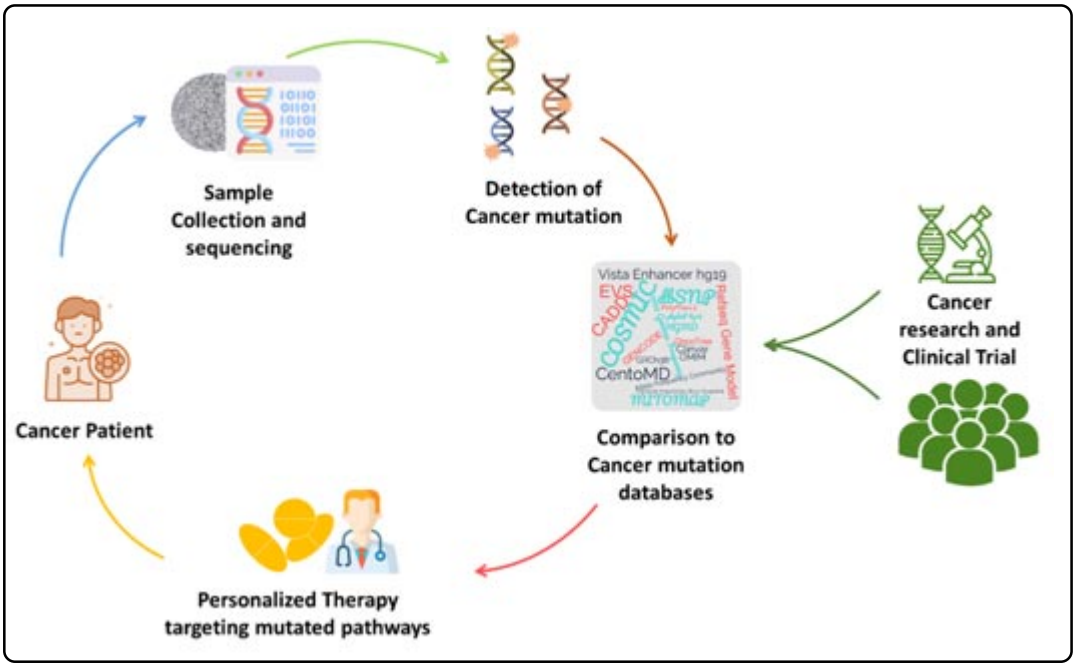

Figure 2: Personalized medicine in Cancer Genomics: The genetic material (DNA/ RNA) from cancer samples are sequenced to detect specific alternations in a cancer patient. Based on the detected aberrations personalized medicines are recommended by specialists to stop the cancer growth.

Metagenomics - Infectious diseases

Considering infectious disease surveillance in public health, knowledge on the causative agent (pathogen) of interest is important. Under standard laboratory practices, this pathogen must be known with a validated test for detection. In case of unknown and unusual infectious diseases due to emerging pathogens, they can be screened against the known suspected pathogens. However, conventional laboratory practices are not always reliable due to evolving pathogenic traits at genetic levels. At such critical exigency, metagenomic investigational methods provide pathogen-agnostic approach to include culturable and non-culturable microbes. Interestingly, studies have reported the efficiency of metagenomics as a diagnostic tool in cases where traditional detection methods for pathogen detection have failed. Foremost application of metagenomics (shotgun) was explored by, Willson et al. and detected presence of Leptospirosis in cerebrospinal fluid (CSF).[24] In another case, shotgun metagenomics identified presence of Abiotrophia defective in culture negative blood and valve samples of endocarditis patient.[25] For other similar studies, investigators employed metagenomics approach in unsolved (culture negative) cases.[26-29] Although pathogen specific molecular tools and serology can confirm the diagnosis/causative agent, the atypical clinical manifestations call for metagenomics approach.

These studies suggest the advantage of metagenomics in identifying non-culturable microbes in presumed sterile samples. This can further be deployed in developing new diagnostic tests, and moreover in developing algorithms to predict the future genetic and etiological instances for the pathogen under consideration.

Detection of antibiotic resistance is the next

engaging application of NGS since gut microbiota majorly comprises of uncultivable bacteria,[30] using metagenomic approach, demonstrated the presence of higher number of antibiotic resistance genes in patients when compared to control, which was otherwise overlooked by culture based methods.[30] Thus metagenomics can be crucial in simultaneous pathogens detection and presence of antibiotic resistance genes.The advantages of NGS are represented in Figure 3.

Metagenomics - Non-infectious diseases

The application of NGS metagenomics goes beyond infectious agent detection. Clinical applications include disease correlation with the host microbiome, viral agent identification in oncology studies and subsequently developing bio-therapeutics. The incomplete clarity/understanding of the microbiome complexity and its involvement on disease pathogenesis has hindered the validation of microbiome-oriented tests in clinical practices.[31] For instance, the infection onset due to Clostridium difficile, an opportunistic pathogen, occurs under altered gut microbiome condition as reported by multiple studies. With applicative approach, management and treatment of Clostridium difficile-associated diseases could be among first clinical application of microbiome NGS.[32] Similarly, metagenomics can be a potential screening tool in discriminating the infectious from the non-infectious illnesses. Several disorders are associated with dysbiotic microbiome such as obesity, diabetes mellitus, cardiovascular diseases and inflammatory bowel disease, and manipulating the microbiome to patient's advantage would require an overview on the microbial landscape.[33]

Moreover, the advancement of NGS into RNA libraries for detection pathogens such as RNA viruses incidentally leads to generation of host gene expression data, i.e. transcriptome analyses (RNA-seq).[34] RNA-seq for gene expression profiling is used at present to characterise infections such as staphylococcal bacteraemia,[35] candidiasis,[36] tuberculosis[37] and Lyme disease.[38] To further this, subjecting the RNA-seq data to machine learning based analyses can be applied for early cancer detection and classification,[39] 2017). Parentheti-cally, WGS approach in identifying the mutated and/ or differentially expressed genes in cancer, data can be looked up for uncovering the viral association with cancer and the subsequent host-virus interactions.[40] Some of these viruses include herpes viruses, papilloma viruses and polyoma viruses, interest-ingly, Merkel cell polyomavirus, now believed to be the cause of Merkel cell carcinoma was first discovered with NGS technology.[41] Metagenomics

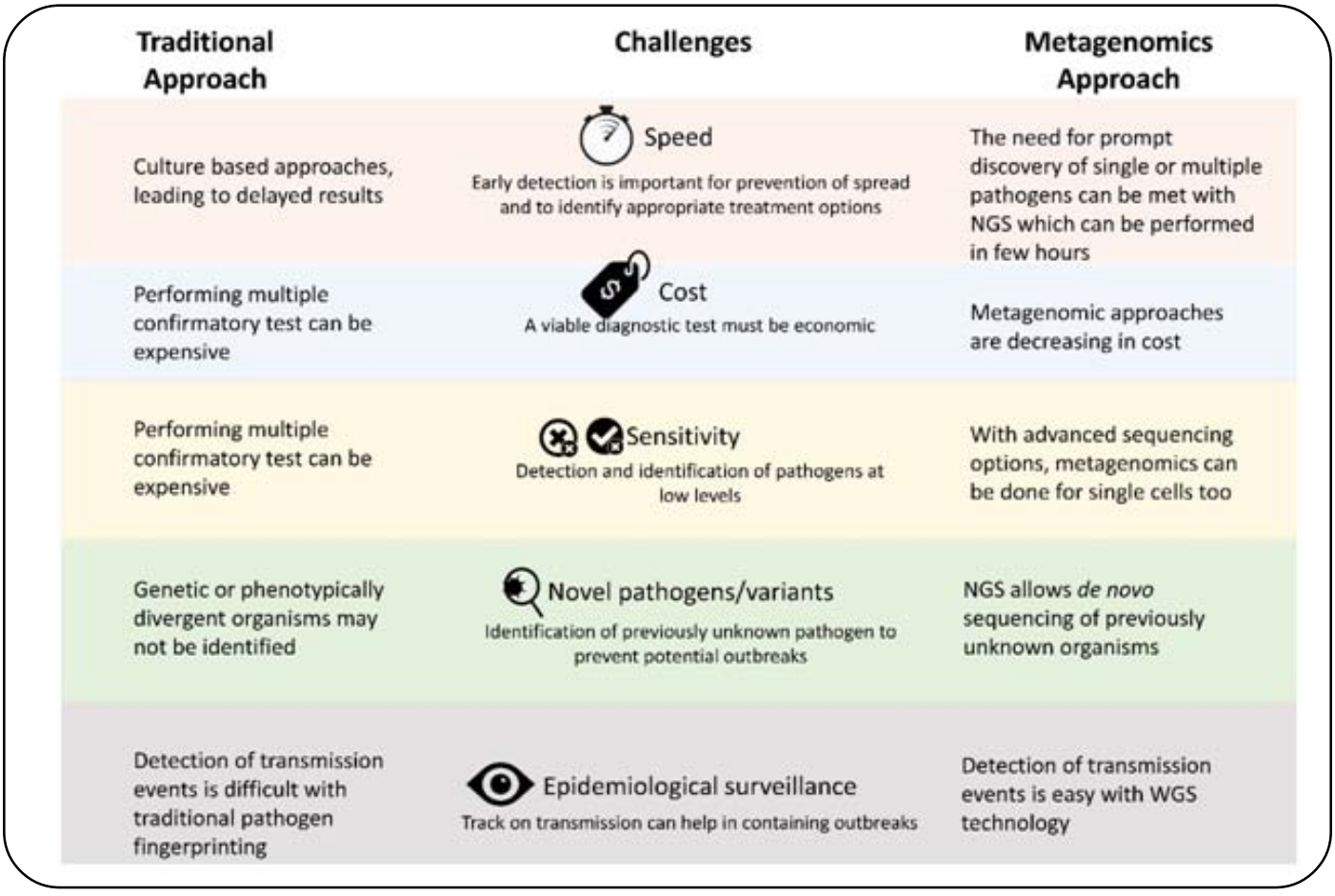

Figure 3: Metagenomics v/s traditional approaches: Challenges in the identification of pathogenic agents and advantages of the metagenomic approaches over traditional approaches.

using shotgun sequencing is a common choice of NGS in clinical metagenomics since it sequences all of the DNA and/or RNA in a given clinical sample. Clinical samples vary significantly in terms of their cellularity (cell-free body fluids or tissues). NGS is an interesting tool in prenatal testing and in identifying mutations to promote diagnosis in pre-symptomatic stages.[42]

Conclusion

With a rise in the urgency of identification of biological causative-agent, NGS has aided DNA/RNA sequencing and enhanced variant/mutation identification in near real time. This allows parallel sequencing of DNA or RNA samples with different lengths as well as whole genome sequencing. Moreover, NGS is applicable for both, cellular and cell-free nucleic acid sequencing - a major advantage over traditional culture-based approaches of pathogen detection. This also broadens the sample type requirement for sequencing, enabling tumor detection using mere liquid biopsy. Additionally, the landscape of microbial population in a given biological niche on the disease status can be explored with metagenomic approach, an important application of NGS. This has furthered our understanding on the role of microbial composition in different infectious as well as systemic diseases. Thus, NGS can provide a comprehensive overview on the disease at genetic level (host), identify causative agent (microbial) and also aid in uncovering the role of the host microbiome in disease pathology.

References:

1. Borry, P. et al. The challenges of the expanded availability of genomic information: an agenda-setting paper. J. Community Genet.9, 103-116 (2018).
2. McKusick, V. A. & Ruddle, F. H. A new discipline, a new name, a new journal. Genomics1, 1-2 (1987).
3. Weissenbach, J. The rise of genomics. C. R. Biol.339, 231-239 (2016).
4. WATSON, J. D. & CRICK, F. H. C. Molecular Structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid. Nature171, 737-738 (1953).
5. Giani, A. M., Gallo, G. R., Gianfranceschi, L. & Formenti, G. Long walk to genomics: History and current approaches to genome sequencing and assembly. Comput. Struct. Biotechnol. J.18, 9-19 (2020).
6. Gomes, A. & Korf, B. Genetic Testing Techniques. in Pediatric Cancer Genetics 47-64 (Elsevier, 2018). doi:10.1016/B978-0-323-48555-5.00005-3
7. Chen, Y.-C., Liu, T., Yu, C.-H., Chiang, T.-Y. & Hwang, C.-C. Effects of GC Bias in Next-Generation-Sequencing Data on De Novo Genome Assembly. PLoS One8, e62856 (2013).
8. Klassen, J. L. & Currie, C. R. Gene fragmentation in bacterial draft genomes: extent, consequences and mitigation. BMC Genomics13, 14 (2012).
9. Payne, A., Holmes, N., Rakyan, V. & Loose, M. BulkVis: a graphical viewer for Oxford nanopore bulk FAST5 files. Bioinformatics35, 2193-2198 (2019).
10. Xiao, T. & Zhou, W. The third generation sequencing: the advanced approach to genetic diseases. Transl. Pediatr.9, 163-173 (2020).
11. Katale, B. Z. et al. Whole genome sequencing of Mycobacterium tuberculosis isolates and clinical outcomes of patients treated for multidrug-resistant tuberculosis in Tanzania. BMC Genomics21, 174 (2020).
12. Murray, S. Challenges of tuberculosis control. Can. Med. Assoc. J.174, 33-34 (2006).
13. Zhang, P. et al. Challenges Faced by Multidrug-Resistant Tuberculosis Patients in Three Financially Affluent Chinese Cities. Risk Manag. Healthc. PolicyVolume 13, 2387-2394 (2020).
14. WHO. Cancer. WHO (2022). Available at: https://www.who.int/news-room/fact-sheets/detail/cancer. (Accessed: 15th February 2022)
15. Sabour, L., Sabour, M. & Ghorbian, S. Clinical Applications of Next-Generation Sequencing in Cancer Diagnosis. Pathol. Oncol. Res.23, 225-234 (2017).
16. Shen, T., Pajaro-Van de Stadt, S. H., Yeat, N. C. & Lin, J. C.-H. Clinical applications of next generation sequencing in cancer: from panels, to exomes, to genomes. Front. Genet.6, (2015).
17. Gambardella, V. et al. Personalized Medicine: Recent Progress in Cancer Therapy. Cancers (Basel).12, 1009 (2020).
18. Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol.15, 579-590 (2017).
19. Bardgett, R. D. & van der Putten, W. H. Belowground biodiversity and ecosystem functioning. Nature515, 505-511 (2014).
20. Kahn, R. A., Fu, H. & Roy, C. R. Cellular hijacking: a common strategy for microbial infection. Trends Biochem. Sci.27, 308-314 (2002).
21. Weinstock, G. M. Genomic approaches to studying the human microbiota. Nature489, 250-256 (2012).

22. Zhao, S. et al. Novel Glycoside Hydrolases Identified by Screening a Chinese Holstein Dairy Cow Rumen-Derived Metagenome Library. Appl. Environ. Microbiol.76, 6701-6705 (2010).
23. Finkbeiner, S. R. et al. Metagenomic Analysis of Human Diarrhea: Viral Detection and Discovery. PLoS Pathog.4, e1000011 (2008).
24. Wilson, M. R. et al. Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing. N. Engl. J. Med.370, 2408-2417 (2014).
25. Fukui, Y. et al. Metagenomic analysis for detecting pathogens in culture-negative infective endocarditis. J. Infect. Chemother.21, 882-884 (2015).
26. Hilton, S. K. et al. Metataxonomic and Metagenomic Approaches vs. Culture-Based Techniques for Clinical Pathology. Front. Microbiol.7, (2016).
27. Pendleton, K. M. et al. Rapid Pathogen Identification in Bacterial Pneumonia Using Real-Time Metagenomics. Am. J. Respir. Crit. Care Med.196, 1610-1612 (2017).
28. Salzberg, S. L. et al. Next-generation sequencing in neuropathologic diagnosis of infections of the nervous system. Neurol. - Neuroimmunol. Neuroinflammation3, e251 (2016).
29. Mongkolrattanothai, K. et al. Neurobrucellosis: Unexpected Answer From Metagenomic Next-Generation Sequencing. J. Pediatric Infect. Dis. Soc. piw066 (2017). doi:10.1093/jpids/piw066
30. Andersen, H. et al. Use of Shotgun Metagenome Sequencing To Detect Fecal Colonization with Multidrug-Resistant Bacteria in Children. J. Clin. Microbiol.54, 1804-1813 (2016).
31. Chiu, C. Y. & Miller, S. A. Clinical metagenomics. Nat. Rev. Genet.20, 341-355 (2019).
32. Samarkos, M., Mastrogianni, E. & Kampouropoulou, O. The role of gut microbiota in Clostridium difficile infection. Eur. J. Intern. Med.50, 28-32 (2018).
33. Boulangé, C. L., Neves, A. L., Chilloux, J., Nicholson, J. K. & Dumas, M.-E. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med.8, 42 (2016).
34. Wang, Z., Gerstein, M. & Snyder, M. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet.10, 57-63 (2009).
35. Ahn, S. H. et al. Gene Expression-Based Classifiers Identify Staphylococcus aureus Infection in Mice and Humans. PLoS One8, e48979 (2013).
36. Zaas, A. K., Aziz, H., Lucas, J., Perfect, J. R. & Ginsburg, G. S. Blood Gene Expression Signatures Predict Invasive Candidiasis. Sci. Transl. Med.2, (2010).
37. Singhania, A. et al. A modular transcriptional signature identifies phenotypic heterogeneity of human tuberculosis infection. Nat. Commun.9, 2308 (2018).
38. Bouquet, J. et al. Longitudinal Transcriptome Analysis Reveals a Sustained Differential Gene Expression Signature in Patients Treated for Acute Lyme Disease. MBio7, (2016).
39. Zhang, Y.-H. et al. Identifying and analyzing different cancer subtypes using RNA-seq data of blood platelets. Oncotarget8, 87494-87511 (2017).
40. Nakagawa, H. & Fujita, M. Whole genome sequencing analysis for cancer genomics and precision medicine. Cancer Sci.109, 513-522 (2018).
41. Feng, H., Shuda, M., Chang, Y. & Moore, P. S. Clonal Integration of a Polyomavirus in Human Merkel Cell Carcinoma. Science (80-.).319, 1096-1100 (2008).
42. Babayan, A. & Pantel, K. Advances in liquid biopsy approaches for early detection and monitoring of cancer. Genome Med.10, 21 (2018).

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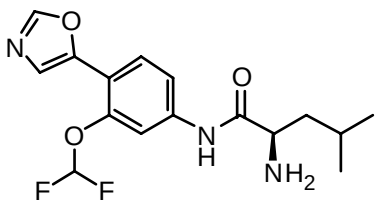
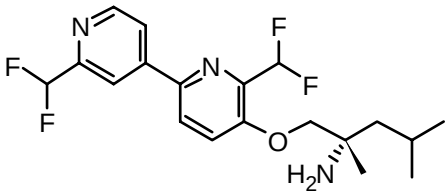
CRIPS Digest

Discovery of Biaryl Alkyl Amides and Biaryl Alkyl Ethers as AAK1 Inhibitors for the Treatment of Neuropathic Pain

Adaptor-associated protein kinase 1(AAK1) is a serine/ threonine kinase, from the family of NUMB associated kinases (NAKs). It regulates the clathrin mediated endocytosis by phosphorylating the Thr-156 of the μ2 subunit of the adaptor protein complex 2 (AP-2). AAK1 is commonly expressed in the spinal cord and brain. It was identified as a potential therapeutic target for the treatment of neuropathic pain based on the screening of 3097 mouse knockout lines in hot-plate and formalin assay. For this, NSAIDs, TCAs, SNRIs, and gabapentinoids are recommended as first-line treatments and weak opioid analgesics such as tramadol and tapentadol are recommended as second-line treatment. A library of clinically used kinase inhibitors was screened from which small repurposed drugs such as baricitinib, momelotinib, lestaurtinib, nintetanib, fedratinib, sunitinib, etc were identified as AAK1 inhibitors. Several other selective inhibitors such as LP-935509, LP-922761, LP-927443, BMS-901715, BMT-090605, BMT-124110 were identified. AAK1 has also been studied as a potential drug target for the treatment of SARS-CoV-2, dengue virus and Ebola virus. The authors carried out the SAR studies of a novel class of bi(hetero)aryl ethers which was inspired from their own previous work on biaryl amide-based compounds, a highly selective and potent AAK1 inhibitor BMS-9861762/ LX-9211 etc. was identified which has entered into phase II human clinical trials for diabetic peripheral neuropathic pain and post-herpetic neuralgia. (J. Med. Chem.2021, 64, 11090; J. Med. Chem. 2022, 65, 4457; J. Med. Chem. 2022, 65, 4534).



Bi(hetero)aryl alkyl amide



BMS-986176/LX9211
Bi(hetero)aryl aryl alkyl ethers

Optimization of TAM16, a Benzofuran That Inhibits the Thioesterase Activity of Pks13; Evaluation toward a Preclinical Candidate for a Novel Antituberculosis Clinical Target.

Tuberculosis (TB) is an infectious disease caused by the bacteria Mycobacterium tuberculosis that mostly affects the lungs. Every year, more than 10 million individuals are diagnosed with tuberculosis, and 1.4 million of them die as a result of the disease. The typical TB treatment plan includes first-line medications isoniazid (INH), rifampicin (R), pyrazinamide (Z), and ethambutol (E) administered over 6 months of directly observed treatment short strategy (DOTS) with a cure rate of 90 to 95 percent. Despite the ability to provide anti-TB treatment quickly (DOTS), the fight against tuberculosis continues since drug resistance is rising. Mycolic acids are vital lipid components of the mycobacterial cell membrane that are required for the survival and pathogenicity of the Mycobacterium genus. Their production is a complex process that necessitates the coordination of approximately 20 enzymes. The clinical use of first-line drugs isoniazid and ethionamide, as well as the preclinical efficacy of previously reported Mmpl3 inhibitors such as indoleamides and adamantylureas, have all demonstrated that inhibiting pathways related to mycolic acid synthesis is a viable approach for anti-TB drug discovery. Polyketide synthase 13 (Pks13) was identified as the main enzyme involved in the final assembly stage of mycolic acid production by Claisen-type condensation. Aggarwal and colleagues have revealed new

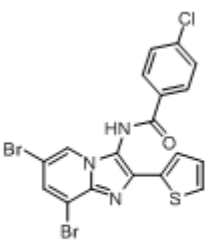
Compound 1
Pks13 IC_{50}: 0.32 μM
H37Rv MIC: 0.08 μM
hERG IC_{50}: 6.9 μM
microsomal clearance: 2.8 mL/min/g

Compound 6
Pks13 IC_{50}: 0.32 μM
H37Rv MIC: 0.67 μM
hERG IC_{50}: >30 μM
microsomal clearance: 2.9 mL/min/g

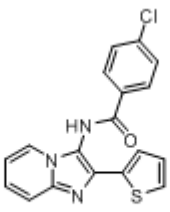
Benzofuran series compounds as Pks13-thioesterase (TE) domain inhibitors of Mycobacterium tuberculosis. Many drugs having lipophilic amine residue interact with the hERG channel and exhibit cardiotoxicity. When the first piperidine moiety containing lead chemical 1 was examined for hERG susceptibility, it demonstrated high potency in vitro Q-patch testing, indicating a risk of cardiotoxicity. (IC_{50}=6.9 μM) The inhibition of Pks13 was dramatically decreased when the piperidine moiety was substituted with a cyclohexyl or phenyl group. Keeping these considerations in mind, other less basic moieties were explored for Pks13 assay, and a species with 2-oxa-6-azaspiro[3.4]-octane moiety demonstrating the best overall features of the series has been identifies. Despite improvements in the hERG liability in vitro, compound 6 (IC_{50} >30 μM) still produced cardiac abnormalities when tested in ex vivo cardiotoxicity models. While there have been improvements, the Cardiac profiler panel found that hERG channel blockage remained a potential hazard. This series of benzofurans has been associated with both direct target binding and off-target cardiovascular harm owing to hERG channel blocking by a lipophilic amine, hence further series development was suspended due to safety concerns. Pks13 is, however, an appealing potential target for antitubercular medicines due to its in vivo activity ability to encourage the establishment of diverse chemotypes.(J. Med. Chem. 2022, 65, 409).

Design and synthesis of imidazo[1,2-a]pyridine-3-carboxamide derivatives as GABA agonists

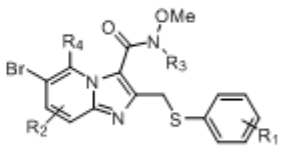
Targeting δ-selectivity for the GABA receptors, many active molecules were reported. Delta subunit containing GABA receptors play crucial role in CNS related diseases for example alcoholism, epilepsy, and major depression disorders. Imidazo[1,2-a]pyridine containing various drugs and drug candidate are already available in the market in which, DS1 and DS2 exhibit significant δ selectivity. DS1 is known drug which possesses positive allosteric modulator activity for GABA which is an inhibitory neurotransmitter, DS1 increases the tonic current in thalamus area of the brain. DS2 is a potent δ-selective agoinst found to have selective α4/6β3δ positive allosteric modulator activity. Recently, in the year 2021 Frolund et al. reported a series of DS2 analogues in order to find potent δ-selective allosteric modulators. Inspired from the GABA agonist DS1 and DS2, an attempt was made to design and synthesized novel imidazo[1,2-a]pyridine-3-carboxamide derivatives. It was proposed that the structural determinants of DS1 and DS2 are modified by introducing functionalization on C-5 position by various alkyl and aryl substitution. At C-2 position instead of thiophene, methyl thiophenol has been introduced. N-methoxy amide group were introduced in order to observe the effect of substitution at C-3 position, where R3 could be H, alkyl or benzyl to achieve bioisosteric replacement. Direct C-5 activation in the case of imidazo[1,2-a]pyridine itself is a challenging task. Metal catalysed C-5 activation is much more troublesome therefore; this position has been less explored. In order to find the significance of C-5 substituted analogues of imidazo[1,2-a]pyridine-3-carboxamide this work has been designed. The substitution at C-5 position may affect the δ selectivity which is required to develop a molecule for targeting CNS related diseases. (J. Med. Chem. 2021, 64, 4730).



DS1 (GABA positive allosteric modulator)



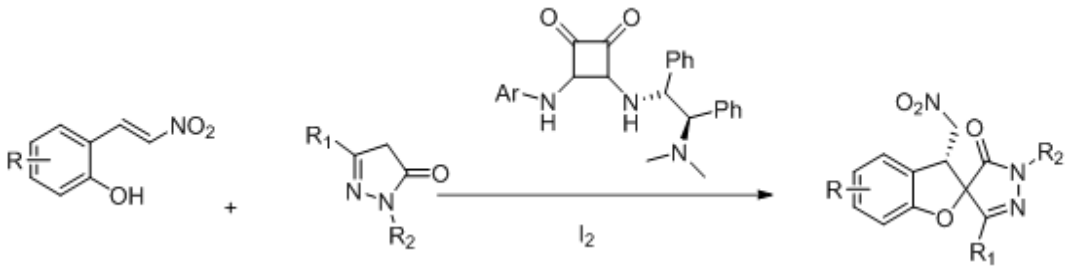
DS2 (GABA positive allosteric modulator)



Designed analogues

Coupled organocatalysis Green synthesis of 2-(azol-1-yl) indoles

The aerobic oxidative cross-coupling of indoles with azoles driven by flavin-iodine-coupled organocatalysis has been developed for the green synthesis of 2-(azol-1-yl) indoles. The coupled organocatalytic system enabled the one-pot three-component synthesis of 2-azolyl-3-thioindoles from indoles, azoles, and thiols in an atom-economical manner by utilizing molecular oxygen as the only sacrificial reagent. In this article development of a simple, convenient, transition metal-free (molecular iodine) catalysed one pot synthesis of 3,5-disubstituted-1,2,4-triazoles. The reaction mechanism involves an initial intermolecular nucleophilic addition (facilitated by I_2) followed by intramolecular nucleophilic cyclisation. It was also demonstrated that 1,1-diaminoazines can act as effective organocatalysts for the formation of phosphorus-carbon bond between biphenylphosphine oxide and an activated alkene (Michael acceptor). These catalysts provide the P-C adducts at a faster rate and with relatively better yields in comparison to the organocatalysts employed earlier.3 Now, our approach is to utilize I2 and 1,1-



diaminoazine in dual catalysis for the formation of heterocycles. (Chem. Comm. 2021, 57, 11717)

Identification of Cryptic Binding Site Using MixMd with standard and Accelerated Molecular Dynamics

Finding and optimizing ligand molecules for a specific binding site is a very efficient method of drug discovery. To do so, it is critical to identify the binding site and comprehend its nature. However, due to the dynamic nature of the protein, which results in numerous conformational states of the binding site, it cannot be easy. When there is a large movement of structures at the binding site, complications arise. Techniques for predicting the binding site based on cosolvent-molecular dynamics have been developed. These strategies take into account the flexibility as well as the effect of the surrounding environment. Mixed Solvent MD (MixMD), SILCS, MDMix, and other approaches fall under this category. The mixed MD approach involved simulating protein in water with probes and detecting the protein surface with the highest probe occupancy. Organic solvents such as isopropanol (IPA), acetonitrile (ACN), pyrimidine (PYR), and others are commonly used as probes. Binding site identification for cryptic sites is a little more difficult. "Cryptic sites are binding sites that are closed in the apostate and open to bind ligands by a structural change." The rearrangement can be as minor as a sidechain rotation or as significant as domain movement." The authors used the MixMD with standard MD and accelerated MD protocol to map the cryptic binding site on the 12 proteins in the current study. The proteins in this list were derived from the Cryptosite dataset. They used five distinct probes: IPA, PYR, ACN, and a set of methylammonium (MAI) + acetate (ACT) ions at the same time. Unbound protein states were employed to test MixMD's competence to introduce conformational changes and map the cryptic site. Using AmberTools and Amber18, they investigated the 12 protein system. This simulation makes use of TIP3P water and the FF14SB forcefield. 12 proteins that are under study were ricin A-chain, adipocyte lipid-binding protein (ALBP), androgen receptor (AR) (ligand-binding domain), secretase (BACE-1), guanylate kinase (kinase domain), lactamase, TIE-2 (kinase domain), protein tyrosine phosphatase 1B (PTP1B, allosteric site), hepatocyte growth factor receptor (c-Met, kinase domain), UDP-N-acetylglucosamine-pyrophosphorylase (UAP1), arylalkylamine N-acetyltransferase (AANAT), and Hsp90. MixMD successfully mapped the system with a wide range of movement, according to the results. For ricin, ALBP, AR, BACE-1, HSP90, Gluanylate kinase, and TIE-2, they were able to identify the binding site and detect suitable conformational sampling.

In the case of BACE-1, where the flaps can close on the binding site, the study was able to facilitate loop movement. Guanylate kinase demonstrated GMP-binding domain mobility, allowing binding site mapping. Furthermore, for guanylate kinase, they utilize charged probes to map the more exposed ATP (ACT, MAI). Although MixMD failed in some cases because whole helices movement was required to open the pocket in those structures. MixMD is thought to have failed due to a short timescale (100ns); another possibility is that the probes were chosen improperly. In conclusion, they stated that there was no consistent probe that mapped well across all of the systems, implying that a variety of probes should be used with MixMD unless first-hand knowledge of the chemical requirements of the desired site is available. (J. Chem. Inf. Model. 2021, 61, 1287).

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NIPER News

(January-March, 2022)

National Girl child day celebrated on 24.01.2022

National Girl Child Day was celebrated on 24.01.2022. The following three Events were organized

1. Poster making competition
2. Essay writing competition
3. A webinar

Prof. Panda, the Director, NIPER, SAS Nagar, Dr. Savita Singh, Ex-Scientist, NIPER, SAS Nagar, Dr. Dipika Bansal, Asstt. Prof. Dept. of Pharmacy Practice, NIPER, SAS Nagar, Ms. Ruheena Priyadarshini, Director, Grazing dreams Infomedia Private Limited, Ms. Uma, Administrative Staff, NIPER, SAS Nagar delivered lectures on this occasion. This was followed by discussion sessions with students as well as with children.

DBT Sponsored Skill Vigyan Training Programme

The TDC-Dosage form (Formulation) centre is conducting two (02) Skill Development Programmes per year, each of 3 Months. The training program modules are skill development for Production/ Manufacturing Chemist & Quality Management System In-charge. Twenty students in each training module are participating in this training programme and these students have been selected on merit basis through the entrance test at all India level. NIPER inaugurated the DBT sponsored Skill Vigyan Training Programme at its TDC-Dosage form (Formulation) Facility in virtual mode on 27-12-2021 and for physical mode training on 14-03-2022 at TDC-Dosage, NIPER.

Prof. Dulal Panda, the Director NIPER, SAS Nagar, Prof. A.K. Bansal, the Dean, and HOD of Pharmaceutics & TDC-Dosage NIPER, Mr. Banoth Rajkumar Naik, Scientist & Skill Vigyan Training coordinator, Dr. Dapinder Kaur Bakshi, Joint Director, PSCST, Mr. J.K. Chandel, Officiating Registrar, NIPER were present in the inaugural programme.

Celebration of Republic Day on 26.01.2022 at NIPER, SAS Nagar

The Institute celebrated 73rd Republic Day with great fervor and enthusiasm. Prof. Dulal Panda, the Director, NIPER, SAS Nagar, unfurled the National Flag followed by recitation of the National Anthem.

In his speech, Prof. Panda offered his heartfelt devotion and respect to the thousands of freedom fighters who laid down their lives in the long struggle for the independence of the country. He mentioned that the Constitution of India gives equal rights to its citizens and sincerely thanked the men and women who framed our Constitution.

He mentioned that our responsibility had increased many folds to generate skilled workforce equipped to take diverse Pharma industry challenges.

Foundation day celebrations at NIPER, SAS Nagar

The institute celebrated its Foundation Day on 15th March 2022 in a felicitous manner under Azadi Ka Amrit Mahotsava (AKAM) celebrations at NIPER convention centre. On this occasion, Prof. S. Anantha Ramakrishna, the Director, Council of Scientific and Industrial Research - Central Scientific Instruments Organization (CSIR-CSIO) was the Chief Guest and Prof. Anil Gulati, Chairman and CEO Pharmazz Inc., Professor Emeritus, Midwestern University, Chicago, USA was the Guest of Honour.

Lecture Series by NIPER faculty

On 21st January, 2022 lecture a series was started by NIPER faculty. The details are as under:

There was a first special Lecture (online) by Prof. Dulal Panda, the Director, NIPER, SAS Nagar. The title of his talk was "Microtubules Targeted Anticancer Drugs Opportunities and Challenges".

Subsequently seven faculty members delivered lectures in this weekly series during this quarter. As a part of this series of lectures, Dr. Rajeev Singh Raghuvanshi, Secretary-cum-Scientific Director, Indian Pharmacopoeia Commission, delivered a lecture. The title of his talk was "Role of Pharmacopoeia in Ensuring Quality of Medicines".

International Women's Day celebrated at NIPER, SAS Nagar

International Women's Day was celebrated on 08.03.2022. Prof. Dulal Panda, the Director of the institute addressed the gathering. He talked about the education part of women. He gladly mentioned that four women scientists got elected for the world Academy of Sciences from India this year which means that women are making a strong mark in every field of science. He wished the audience all the best on this great day.

There was a talk given by Dr. Harsimrat Kalsi, Senior Consultant Physiotherapist, Touch Clinic, Mohali. It was followed by cultural programme.

Discussion during National Girl Child day on 24th January 2022

DBT's Skill Vigyan Training programme 17th March, 2022

NIPER, S.A.S. Nagar signed MoU with Dept. of Industries Himachal Pradesh (March 24, 2022)

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