Current and Emerging Therapeutic Modalities in Drug Development

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Abstract

Over the past few decades, enhanced understanding of complex disease biology has unraveled plethora of novel targets for drug discovery. With an increasing focus of delivering targeted therapies, drug development companies have adapted a wider chemical toolbox for strategic drug design and clinical success. In this article, we highlight the current and new chemical modalities considered for drug discovery including: small molecules, antibody-drug conjugates, RNA-based therapies, protein degraders, and gene therapy.

Key Words:*Drug Modalities, Small Molecules, Antibodies, RNA-based therapy, PROTACs, Gene Therapy*

Introduction

Emerging landscape of information from Multi-Omics Big Data sets (DNA-Seq, RNA-Seq and Proteomics) has led to deeper understanding of diseases such as cancer, cardiovascular disease and neurodegenerative diseases[1-3]. This effort surfaced drug targets, pathways and mechanisms that are patient-centric and clinically relevant thereby harnessing a need to develop novel therapies. For decades, the conventional approach for drug discovery has been modulation of protein activity with small molecules. Although this strategy has proven high clinical success with a number of FDA approved drugs in the market, the absence of ligandable surface area on certain proteins and discovery of disease-relevant RNA and DNA as drug candidates poses a great limitation to this approach. Nevertheless, in the recent years the field has made remarkable strides in advancing novel chemical strategies for delivering clinically effective therapeutics (Figure 1). Biologics, most notably engineered antibodies, are being developed for challenging disease indications. Targeted protein degradation using PROTACs (PROteolysis Targeting Chimeras) is pursued as a ground-breaking approach for targeting the "undruggable" proteome. RNA-directed drugs, including small-interfering RNA (siRNA) and anti-sense oligonucleotides (ASOs) are cutting-edge for RNA-based therapies. Gene editing technologies like CRISPR offer the promise of gene therapy by DNA modulation. Together, these multi-pronged approaches focus on delivering novel medicine with the vision of curing diseases rather than treating symptoms. This report elaborates on these modalities and highlights the recent progress made in the field.

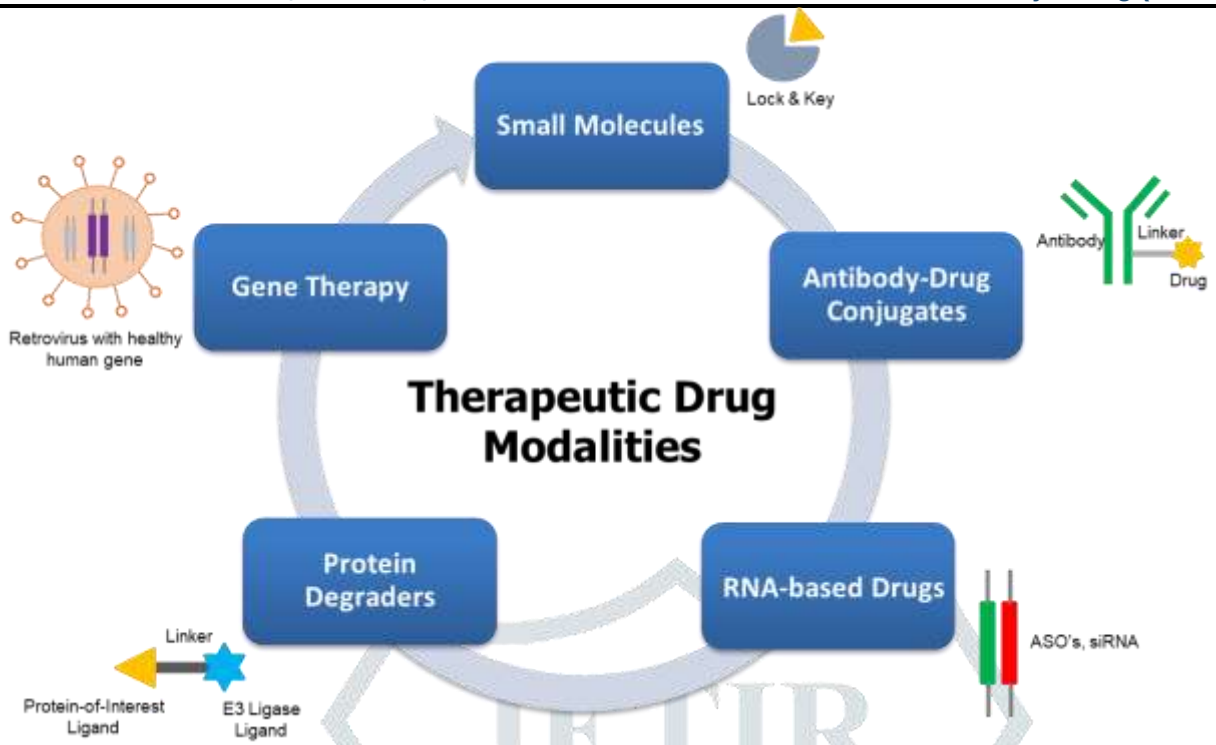

Figure 1: Illustration of existing and emerging drug modalities adapted by pharmaceutical industryfor delivery of targeted therapies

Small Molecules

The pharmacology definition of a "small molecule" is a chemical entity, an organic compound that has low molecular weight (< 900 Daltons) and a size in the order of 1nM. These molecules bind to specific enzyme or receptor pockets on the proteins by lock and key mechanism and thereby alter the activity or function of the target (Small molecule - Wikipedia). Small molecules represent the most widely used approach for drug discovery, their high FDA approval rates in the market highlights the success of this strategy in drug development[4,5]. Infact, about 90% of pharmaceuticals in the market are small molecule drugs. There are three major classes of small molecule drugs: inhibitors, activators and protein-protein interaction disruptors (PPIs). Small molecule inhibitors and activators "turn off" or "turn on" protein activity respectively, while PPIs physically disrupt the interaction between two proteins. Mechanistically, the mode-of-binding of small molecules can be allosteric, orthosteric or covalent. Some of the major advantages of small molecules over other drug modalities are that they offer superior oral bioavailability, relatively easy to synthesize, scalable and highly profitable. Nevertheless, the inability to bind to large surface areas, and absence of ligandable pockets on some proteins pose limitations to this strategy.

Antibody-Drug Conjugates

In the past three to four decades, development of targeted therapeutics, particularly monoclonal antibodies (mABs) for treatment of cancers and immunological disorders have been attracting a lot of interest. Muromonab-CD3 (OKT-3), a murine antibody is the first FDA approved and marketed drug for human therapeutic use [6]. However, the drawbacks associated with the murine antibodies including induction of immunological reactions and reduced human half-lives has led to development of humanized versions that

exhibit a greater probability of success [7]. Adalimumab is the first fully humanized antibody approved for rheumatoid arthritis in 2002 and since then, a number of molecules have been marketed or are in clinical development [8]. Antibody-drug conjugates (ADCs) are an emerging class of therapeutics for cancer. This approach involves combination of chemotherapy and immunotherapy, where a mAB is conjugated to a small molecule via a chemical linker. The concept herein is generation of a hybrid molecule that essentially combines the target specificity of an antibody to bind to cancer cells, and potency of the cytotoxic payload (small molecule) to promote cell killing. ADC's hereby augment the main challenge of chemotherapy associated with therapeutic window as they empower selective delivery of drugs to tumor cells while sparing healthy cells resulting in low systemic toxicity [9]. Few examples of FDA-approved ADC's for oncogenic indications include Adcertis, Mylotarg and Kadcyla are few examples of FDA approved ADC's for oncogenic indications [10]. Till date, there are over 60 ADCs in early to late clinical trials signifying the success of this approach[10].

RNA-based therapeutics

Anti-sense oligonucleotide technology is receiving popularity as a new therapeutic modality for treatment of several genetic diseases[11]. The concept here is to design an antisense oligonucleotide complementary to the disease-relevant mRNA, that when introduced into cells binds to target mRNA and consequently, disrupts protein expression. There are diverse mechanisms by which anti-sense oligonucleotide-based drugs ultimately result in protein abrogation, and these include: target RNA degradation (siRNA or shRNA, RNAase H-dependent degradation), translation interference (Morpholinos, MicroRNAs) and pre-mRNA processing like splicing (ESSENCE, TOESh) or polyadenylation (IPA Activation, U1 interference)[12] . Although this strategy has existed for a long time and offers advantages like relatively simple chemical synthesis, the high molecular weight of these molecules, and existence of multiple negative charges poses a significant challenge with cell permeability. Over the past two decades however, the field has made tremendous progress to improve the pharmacokinetic and pharmacodynamic properties of these agents to particularly overcome limitations like intracellular drug delivery, stability and immune response activation [13]. FDA-approvals for RNA-based drugs namely, Onpattro and GIVLAARI demonstrate progress made in the clinic [10,14]. Furthermore, a number of these drugs have been nominated for clinical trials towards treatment of disease indications such as neuropathy, hypercholesterolemia, cancer, ebola, and hepatitis-B [10,12].

PRO teolysis Targeting Chimeras (PROTACs)

Protein degradation by PROTACs is a cutting-edge strategy for abrogation of "undruggable" targets, such as transcription factors and non-enzymatic proteins. Mechanistically, these molecules utilize an event-driven MOA (mode-of-action) to hijack the ubiquitin-proteasome system leading to targeted protein degradation. A PROTAC is a hetero bifunctional molecule that contains a ligand for binding to E3 ubiquitin ligase (E3 ligand), a linker, and another ligand that recruits protein of interest (POI ligand). It forms a ternary complex by engaging both target protein and E3 ligase simultaneously, and by doing so brings the protein in close proximity with E3 ligase for subsequent ubiquitination and degradation by proteosomes. The first generation of PROTACs were reported by Crews and Deshaies group in 2001. The team demonstrated successful development of PROTACs for targets like methionine aminopeptidase-2 (MetAp-2), androgen (AR) and estrogen (ER receptor) [15-17]. Although these molecules induced specific target degradation, some of the drawbacks of these early generation PROTACs due to peptidic nature of E3 ligand were poor cell permeability and weak cellular activity. However, the field revolutionized with the discovery of small molecule-based E3 ligands. The first all small moleculePROTAC published in 2008, is an AR degrader

that used Nutlin (MDM2-p53 protein-protein inhibitor) as E3 ligand[18,19]. In the later years, CRBN or VHL E3 recruiting ligands were used to develop highly potent cell permeable PROTACs with drug like properties for several targets [20-23]. This achievement marks a huge milestone for the strategy as it highlights the promise of this approach for therapeutic discovery. One of the major focus areas as this technology evolves towards development of clinically effective PROTACs, is addressing some of its critical challenges including: high molecular weight, toxicity, poor stability and biodistribution in tissues. In fact, examples such as ARV-101 (AR-PROTAC) and ARV-471 (ER-PROTAC) that are in phase-1 clinical trials, illustrate the potential of this strategy to provide cures [10].

Gene Therapy

Gene therapy is an ability to make corrections to the mutated diseased gene through site-specific modifications leading to a therapeutic outcome. This strategy offers a huge potential towards treatment of genetic diseases like cystic fibrosis, hemophilia, muscular dystrophy, sickle cell anemia, cancer, and certain viral infections such as AIDS [24-26]. Conceptually, using recombinant DNA technology approaches a healthy gene of interest is inserted into a vector (preferably retroviral) which is then introduced into patient's cells (blood, stem cells, tumor, liver) as a drug to treat the disease [25,26]. Although, this strategy has been in research for at least five decades with over 2900 clinical trials in phase-1 (Gene therapy - Wikipedia), groundbreaking discoveries such as use of genetically engineered T-cells or hematopoietic stem cells and CRISPR-Cas9 technologies have significantly advanced clinical success of this therapeutic approach. In 2017, FDA approved Kymriah (Novartis), a chimeric antigen receptor T cell therapy ("CAR-T") as the first gene-therapy based drug for treatment of lymphoblastic leukemia [10]. CAR-T cell therapy is an immunotherapy approach where immune cells (T lymphocytes) of the patient are genetically engineered to recognize and attack the tumor T cells [25,27-31]. Since then, there have been three more FDA approvals for gene therapy and more than 700 therapies in clinical trials [10,32]. Gene editing technologies like CRISPR-Cas9 that have an ability to correct human genome open doors for this modality as it offers the promise of precision medicine [33,34].

Conclusion

For decades now, a "disease-relevant target" if often considered a protein. Of the 400 protein targets for which we have FDA-approved drugs, about 90% of them are enzymes, transporters, GPCRs, voltage gated ion channels and nuclear receptors that have reasonably high chemical tractability and hence easily druggable with existing modalities [35,36]. By far, the most classical approach to therapeutically drug this target class has been with small molecules. In general, small molecules offer several advantages including oral bioavailability, broad tissue distribution and relatively easier and faster synthesis routes. However, their major limitation has been inability to drug certain bonafide targets like transcription factors that lack hydrophobic binding pockets (example: c-Myc) or proteins with unique mode-of action (example: Ras and Tau). Furthermore, emerging knowledge around biology & complexity of disease and resistance mechanisms is changing the target landscape beyond just proteins and acknowledging RNA and DNA as potential classes of drug targets. It is approximated that current therapies can target only 13% of the diseased target class suggesting that about 85% of the target space remains "undrugged"[35]. To address this, the drug development industry has adapted a multi-faceted approach using novel chemical modalities tailored specifically for each target to enhance therapeutic success, and in this manuscript, we have highlighted illustrative examples and milestones achieved with these modalities.

Acknowledgements

The author wish to thank research laboratory, Department of Biotechnology, Andhra University, Visakhapatnam for Provided necessary laboratory facilities during this entire research work. I express sense

of gratitude and indebtedness to my beloved research supervisors for the patience and affection and which have helped me a lot completing the task.

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