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Computational approaches in design of nucleic acid-based therapeutics

Mark Sherman[1] and Lydia Contreras[2]

Recent advances in computational and experimental methods have led to novel avenues for therapeutic development. Utilization of nucleic acids as therapeutic agents and/or targets has been recently gaining attention due to their potential as high-affinity, selective molecular building blocks for various therapies. Notably, development of computational algorithms for predicting accessible RNA binding sites, identifying therapeutic target sequences, modeling delivery into tissues, and designing binding aptamers have enhanced therapeutic potential for this new drug category. Here, we review trends in drug development within the pharmaceutical industry and ways by which nucleic acid-based drugs have arisen as effective therapeutic candidates. In particular, we focus on computational and experimental approaches to nucleic acid-based drug design, commenting on challenges and outlooks for future applications.

Addresses
[1] Cell and Molecular Biology Graduate Program, University of Texas at Austin, 100 E. 24th Street, A6500, Austin, TX 78712, USA
[2] McKetta Department of Chemical Engineering, University of Texas at Austin, 200 E. Dean Keeton St., Stop C0400, Austin, TX 78712, USA

Corresponding author: Contreras, Lydia (lcontrer@utexas.edu)

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Introduction

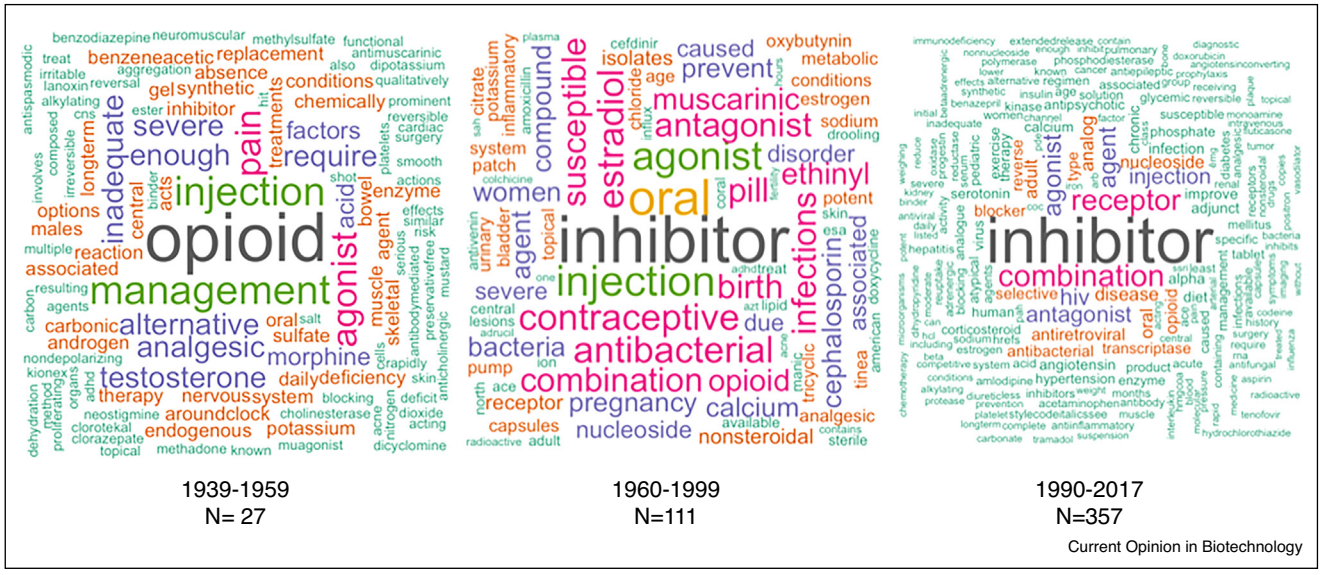
Over almost 80 years of FDA regulation, the field of drug discovery and development has transformed its focus from pain management of disease symptoms to systematic identification of causative factors and targeted mediation (Figure 1). Early pharmaceutical development stemmed from the isolation and concentration of active agents from natural products by chance discovery. Although the therapeutic mechanisms of these substances were unclear at the time, most contemporary drug development efforts aim to target specific disease-associated proteins. The majority of approaches involve screening small molecule libraries for target protein inhibition that lead to therapeutic effects. While these efforts have resulted in hundreds of new drug entities, the journey to approval for the average drug is increasingly costly [1••]. The rising cost of drug development can, in part, be attributed to increased screening efforts due to low hit rates and to misdirected investigations based on proteins that show no interactions with current candidate drug molecule libraries; other reasons include the high probability of failure during the scale-up process and the escalating demand by regulatory bodies for more safety and efficacy data prior to approval [1••,2]. Furthermore, many diseases simply cannot be influenced by small molecules due to the inability to identify a binding pocket in key proteins underlying disease pathways to serve as a suitable drug target. In addition, challenges to small molecules arise from the possibility that disease phenotypes are caused by mechanisms upstream of protein production, such as improper gene splicing, without a suitable small molecule target.

In recent years, drug development efforts have become more sophisticated, relying less on large-scale screening of chemical compounds and more on targeted development of drugs toward specific molecular entities. Modern drug development pipelines attempt to target a wider spectrum of molecules beyond traditional enzyme targets, including metabolites [3], protein–protein interactions [4], and nucleic acids [5]. These advances have enabled treatment of various diseases through the development and engineering of new vaccines (with over 30 active vaccine-associated clinical trials currently underway (clinicaltrials.gov)), peptide therapeutics such as Parsabiv [6], nucleotide prodrugs such as Vosevi [7], as well as a variety of antibody-based technologies including antibody–drug conjugates (used in Mylotarg) [8,9], phage display (used in development of Humira) [10,11•,12], and radiolabelled diagnostic antibodies as used in Netspot [13–15].

Incentives and challenges to nucleic-acid therapeutic development

While protein-based therapeutics (antibodies, recombinant proteins, and peptides) have made up over a quarter of FDA approved drugs since 2015 (FDA.gov), they have relatively a short shelf life, require refrigerated transport, and rely on live organisms for production, making manufacturing them expensive, contamination prone, and variable across batches [16••]. In response to these shortcomings, recent efforts have been made toward development of nucleic acid-based drugs, offering the key advantage of chemical manufacturing. As such, they

Figure 1



An analysis of indications and usage descriptions from FDA approved drug labels across three time periods from 1939 to 2017. Between 1939 and 1959, approved drugs appear to be associated with pain management and symptomatic relief. Between 1960 and 1989, small molecule inhibitors appear to become mainstream and birth control is developed. Between 1990 and 2017, inhibition continued to be a major theme for pharmaceutical development and drugs became more specific toward particular diseases, including viruses such as HIV. During the last 30 years, the emergence of nucleic acids as targets (nucleosides) also represents a newer phenomenon. Data generated by an analysis on a collection of drug labels through U.S. National Library of Medicine's DailyMed website.

maintain a relatively long half life, are easily transportable, are consistent from batch to batch, and have the potential to introduce new therapeutic chemistries [17] to the current, inhibitor-dominated field (Figure 1). For instance, DNA has been identified as a target for drug development [18] for its gene therapy ability via manipulation of gene expression or editing of defective genes [19•]. Likewise, RNA variants have been implicated in a variety of disease phenotypes such as Spinal Muscular Atrophy (SMA) [20], Duchenne Muscular Dystrophy (DMD) [21,22••], Alzheimer's Disease, and cancer [23,24•]. Therefore, development of nucleic acid-based drugs hold promise to provide high affinity and high specificity entities for therapeutic applications; however, progress in this field has been limited by challenges in delivery to specific tissues, a very active area of research (discussed later) [25–31].

In 2016, three nucleic acid-based therapies gained FDA approval: Exondys-51 [22••] (for treatment of DMD), Spinraza [20] (for treatment of SMA), and Defitelio [32] (for treatment of renal or pulmonary dysfunction). Both Spinraza and Exondys-51 are antisense oligonucleotides that therapeutically influence splicing patterns of diseased proteins. The mechanism for Defitelio has not been elucidated [32]. Several other RNA-based or RNA-targeting drugs are currently in clinical trials (Table 1). The majority of these candidate drugs involve infusion of chimeric antigen receptor (CAR) T cells while others are siRNA (small interfering RNA), shRNA (small hairpin RNA), and mRNA (messenger RNA) based. CAR T cells are derived from the patients own T cells which are genetically modified *ex vivo* and introduced back into the body with enhanced therapeutic functions. Currently approved RNA-based drugs are administered as local injections or systemic infusions (Table 1). As research in nucleic acid-based therapeutics progresses, it holds promise to develop drugs that can not only inhibit action of a protein, but could alter gene expression and metabolic flux through development of aptamers (RNA analogs of antibodies), prodrugs, siRNAs, miRNAs, and new vaccines with more efficient modes of delivery.

Current strategies toward aptamer-based therapeutic development

Due to their ability to bind to diverse targets such as ions, dyes, amino acids, RNAs, oligosaccharides, antibodies, and cells, aptamers have become the main focus of nucleic acid-based therapeutic development [33]. Aptamers are short, single-stranded DNA or RNA oligonucleotides that bind to a variety of target molecules with high affinity and specificity, dependent on their tertiary structure [34]. The majority of DNA and RNA aptamers are experimentally developed via Systematic Evolution of Ligands by Exponential Enrichment (SELEX)