Computer-Aided Drug Discovery for SMA

Subjects: Genetics & Heredity | Mathematical & Computational Biology
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Spinal muscular atrophy (SMA), one of the leading inherited causes of child mortality, is a rare neuromuscular disease arising from loss-of-function mutations of the survival motor neuron 1 (SMN1) gene, which encodes the SMN protein. When lacking the SMN protein in neurons, patients suffer from muscle weakness and atrophy, and in the severe cases, respiratory failure and death. Several therapeutic approaches show promise with human testing and three medications have been approved by the U.S. Food and Drug Administration (FDA) to date. Despite the shown promise of these approved therapies, there are some crucial limitations, one of the most important being the cost. The FDA-approved drugs are high-priced and are shortlisted among the most expensive treatments in the world. The price is still far beyond affordable and may serve as a burden for patients. The blooming of the biomedical data and advancement of computational approaches have opened new possibilities for SMA therapeutic development.

Keywords: drug discovery ; drug therapy ; spinal muscular atrophy ; SMA ; neuromuscular disorder ; computational aided drug discovery ; in silico drug repurposing ; artificial intelligence

1. Introduction

Spinal muscular atrophy (SMA) is a rare, progressive neuromuscular disease (NMD), arising from loss-of-function mutations of survival motor neuron 1 (*SMN1*) gene. It is one of the leading inherited causes of infant and early childhood mortality [1][2]. More than 95% of patients struggle from the homozygous deletion of the *SMN1* gene, which is responsible for the encoding of the SMN protein [3]. Consequently, this leads to insufficient SMN protein in neurons, resulting muscle weakness and atrophy, and in severe cases, respiratory failure and death [4]. The severity of SMA, from mild to severe, depends on the presence of the level of SMN protein [5], reflecting an inverse correlation.

Treatment options for SMA are limited and palliative in nature. Even with the remarkable results of approved drugs, the limitations, such as high cost, unknown long-term effects and side effects of the treatments, hinder the success of treating SMA patients. To date, three medications have been approved by the U.S. Food and Drug Administration (FDA) for SMA, which are nusinersen (Spinraza®) from Biogen, onasemnogene abeparvovec-xioi (Zolgensma®) from Novartis and recently approved risdiplam (Evrysdi™). However, the cost of the former two therapies are astronomical in nature [6][7][8], while for the latter drug, which is in the early stage from the announcement of FDA approval, the cost has yet to be established. Besides the high cost of the treatment, the challenging drug administration for the patients with scoliosis and/or spinal deformity may require sophisticated personnel. As scoliosis is a general symptom of SMA patients, most patients do not acquire the maximal benefits from the current treatments. Several promising therapeutic approaches are currently being developed; some are at different stages of clinical trials. Despite this, the medical cost is still far beyond the affordability of the general populace.

With the advancement of computational approaches, next generation therapeutics may provide a rapid and less expensive access to new treatment. Researchers, nowadays, are gaining the advantage of computational technologies, using genomics, transcriptomics and proteomics approaches to study biological interactions that are crucial for disease pathogenesis and development of new therapies. In addition, the structural analysis on the missense mutations in SMN1 protein served as a platform to understand the role of the SMN protein in SMA from the perspective of the molecular structural impact towards drug design. Furthermore, artificial intelligence (AI), machine learning (ML) and/or deep learning (DL) have shifted from hype to hope in the pharmaceutical industry due to increased research and development (R&D) cost and reduced success and efficiency rate in drug discovery. Owing to the incorporation of genomics and biochemical information, AI serves as an ‘Open Target’ platform for the prediction of therapeutic targets, which has been successfully applied to amyotrophic lateral sclerosis (ALS), one of the human neurodegenerative diseases [9]. Although there are no drug discovery studies utilizing this technology, this AI-assisted implementation may offer a future hope for SMA patients.

2. Spinal Muscular Atrophy (SMA)

SMA is a monogenic autosomal recessive genetic disorder characterized by the degeneration of alpha motor neurons (α-MNs) located in the anterior horn of the spinal cord [4][10]. The progressive destruction of α-MNs, which is responsible for initiating the muscle contraction, leads to symmetrical muscle weakness and atrophy [11][12][13]. The primary manifestations of this disease ultimately result in paralysis and often death in severe cases. Proximal muscles, specifically the lower muscles, are affected first, then the upper extremities [7][14]. SMA has a unique genetic background, as the change of functional loss in SMA peaks at the onset of the disease followed by progressive worsening condition [15].

SMA is the leading genetic cause of infant mortality globally [16][17] and the second most common fatal autosomal recessive disorder after cystic fibrosis [18][19]. It occurs with an estimated pan-ethnic incidence of 1 in 6000–10,000 live births and a carrier frequency of 1 in 40–60 [20][21][22]. As of September 2015, a total of 4526 patients are registered under TREAT-NMD, an international network for the neuromuscular field (https://treat-nmd.org/about-the-treat-nmd-network/ (accessed on 1 April 2020)).

2.1. Disease Etiology

About 95% of SMA cases [23][24] are caused by a homologous deletion or mutation of the survival motor neuron 1 (*SMN1*) gene on chromosome 5q13, which is the blueprint for the SMN protein [3]. The *SMN1* is highly conserved and presents as a single copy in the genome of all eukaryotic organisms [25][26]. A normal individual has two forms of the *SMN* gene, which are telomeric *SMN1* and its paralog, centromeric *SMN2* [4][27]. Both genes are nearly identical, with only a difference in five base pairs. However, the base pair differences do not alter the amino acid sequence, and they encode the same SMN protein.

The *SMN1* gene produces full-length, functional SMN (FL-SMN) protein. A synonymous C-to-T base substitution (c.840C > T) at the position 6 of *SMN2* exon 7 disrupts the proper splicing and leads to a majority (~90%) of exon 7-skipped transcript (Δ7-transcript) [27][28][29][30]. Subsequent translation of such transcript results in a truncated and unstable SMN protein [16][31]. Only ~5–10% FL-SMN protein will be produced by the *SMN2* gene, whereas patients with any form of SMA lack a functioning *SMN1* gene and only depend on the *SMN2* gene. Therefore, they are in a condition of deficiency with regards to SMN protein production, and thus, they lead to a loss of motor neurons in the spinal cord.

There are five types of SMA **(Figure 1)**, which are known as SMA type 0, I, II, III and IV. The copy number of *SMN2* gene modifies the severity of the disease phenotype as a high number of *SMN2* copies is related to milder phenotypes [30][32]. For instance, SMA type I patients generally have one or two *SMN2* copies, while SMA type III/IV patients have more than four copies [33][34]. Nevertheless, this inverse relationship is not always true, as a few patients with two *SMN2* copies showed milder SMA phenotypes, while there have also been patients with three *SMN2* copies that have been defined as type I [20][35][36][37]. Lacking either one *SMN* gene leads to low levels of SMN protein, though this still allows embryonic development and usually occurs in SMA carriers. Nevertheless, there are no individuals with neither *SMN* genes, which mean homologous loss, as it is hypothesized to be an embryonic lethal condition [16][38][39]. Notably, SMA patients can be classified into five clinical types based on age of onset and level of motor function [40].

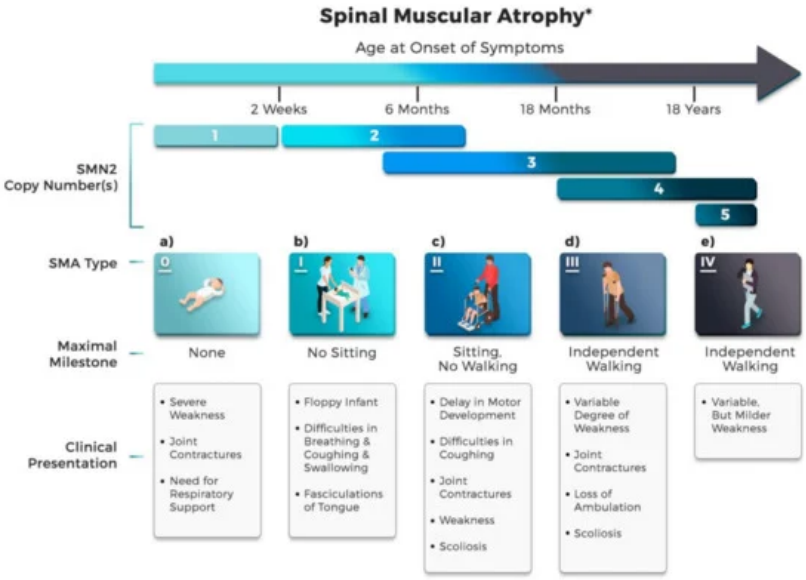

Figure 1. Classification of spinal muscular atrophy (SMA) sub-types. * All SMA patients, regardless of the subtypes, have no functional copies of survival motor neuron 1 (*SMN1*). SMA can be classified into five types (0-IV) ranging from the most

severe form to a milder form. (**a**) Type 0 is the most severe form and in-utero onset. They normally have limited life expectancy. (**b**) Type I infants display clinical symptoms at birth or by the age of six months. They never develop the ability to sit and if no intervention is provided results in death by two years. (**c**) Type II patients are diagnosed within six to 18 months of age and they do develop the ability to sit but they never walk unaided. However, they are able to survive well into adulthood. (**d**) Type III can be further classified into IIIa (onset between 18 months to three years old) and IIIb (onset between ages of three to 30 years old). They have a normal life expectancy. (**e**) Type IV is the mildest form and adult-onset. Patients with type IV have a normal life expectancy.

2.2. SMN Protein

Mutation events of the *SMN1* gene that encodes the SMN protein are predominantly linked to SMA disease [41]. Understanding the molecular structural of SMN protein is important and helpful in the molecular pathogenesis of SMA. However, as of January 2021, there is still no FL-SMN protein structure in Protein Data Bank (PDB; https://www.rcsb.org/ (accessed on 29 January 2021)) and only have SMN-related structures. The SMN, a 38-kDa protein, is ubiquitously expressed in both nucleus and cytoplasm [42] in particular, having a high concentration in motor neurons of the spinal cord and relatively less in lymphocytes and fibroblasts [43]. Human SMN protein is coded by eight exons [44] and it consists of 294 amino acids and harbors several functional domains, including a basic/lysine (K)-rich region, a Tudor domain, a proline (P)-rich region and a tyrosine-glycine rich (YG)-box (**Figure 2**). Those functional domains are highly conserved from yeast to human and play important roles in the motor system as well as intracellular processes [45].

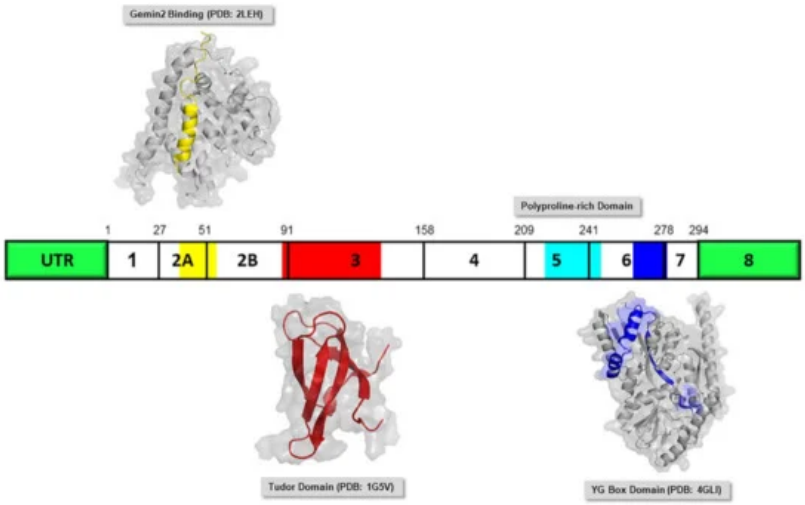

Figure 2. Diagrammatic representation of the full length SMN (FL-SMN) protein with its respective available protein structure. The number of exons is indicated within the boxes of SMN protein diagram with the number of the last amino acid residue of each exon, indicated above. The PDB structure of the domains are illustrated in the same color that overlaid in the SMN protein diagram indicating the location of the respective domains (Gemin2 Binding: yellow; Tudor Domain: red; Polyproline-rich Domain: cyan; YG Box Domain: blue). Abbreviation: UTR, untranslated region.

The Gemin2 binding domain (Ge2BD), coded by exon 2 and located near the N-terminus, is highly conserved among SMN-containing eukaryotes, suggesting the important role of SMN–Gemin2 interaction [46][47]. A central Tudor domain, coded by exon 3 of SMN, facilitates the protein-protein interactions [48]. A most conserved segment near the C-terminus [47], referred to as the 'YG-box,' is responsible for oligomerization, which is crucial for the function of SMN and interaction between SMN with Gemins and Sm proteins. Given the SMN protein's critical role in the biogenesis of snRNPs, SMA patients fully depend on *SMN2* gene to compensate the loss of the *SMN1* gene for the production of the SMN protein. However, a relatively low amount of functional SMN protein is produced while the translated product of aberrant splicing event, termed SMNΔ7 (only consists of 282 residues), is unstable and rapidly degrades [49].

3. Current Drug of SMA - Early Success

It is well known that the disease severity is related to the SMN protein levels, and thus, increasing SMN production has been a major SMA drug discovery strategy [50]. Multiple mechanisms have been targeted to drive higher expression of the full length SMN protein, either from the *SMN2* gene or from the exogenously restored *SMN1* gene [25][51]. The SMN protein was suggested to play a crucial role in neurons and muscle [52][53][54]; hence, SMN-independent therapies that provide neuroprotection or slowing down or halting the events due to the effects of SMN depletion could be an alternative for SMA [55]. Out of 1167 US Food and Drug Administration (FDA)-approved drugs (as of March 2020), there are only

three drugs approved for the treatment of SMA, which are nusinersen (first approved in late 2016), onasemnogene abeparvovec (approved in 2019) and risdiplam (recently approved in August 2020) (**Figure 3**).

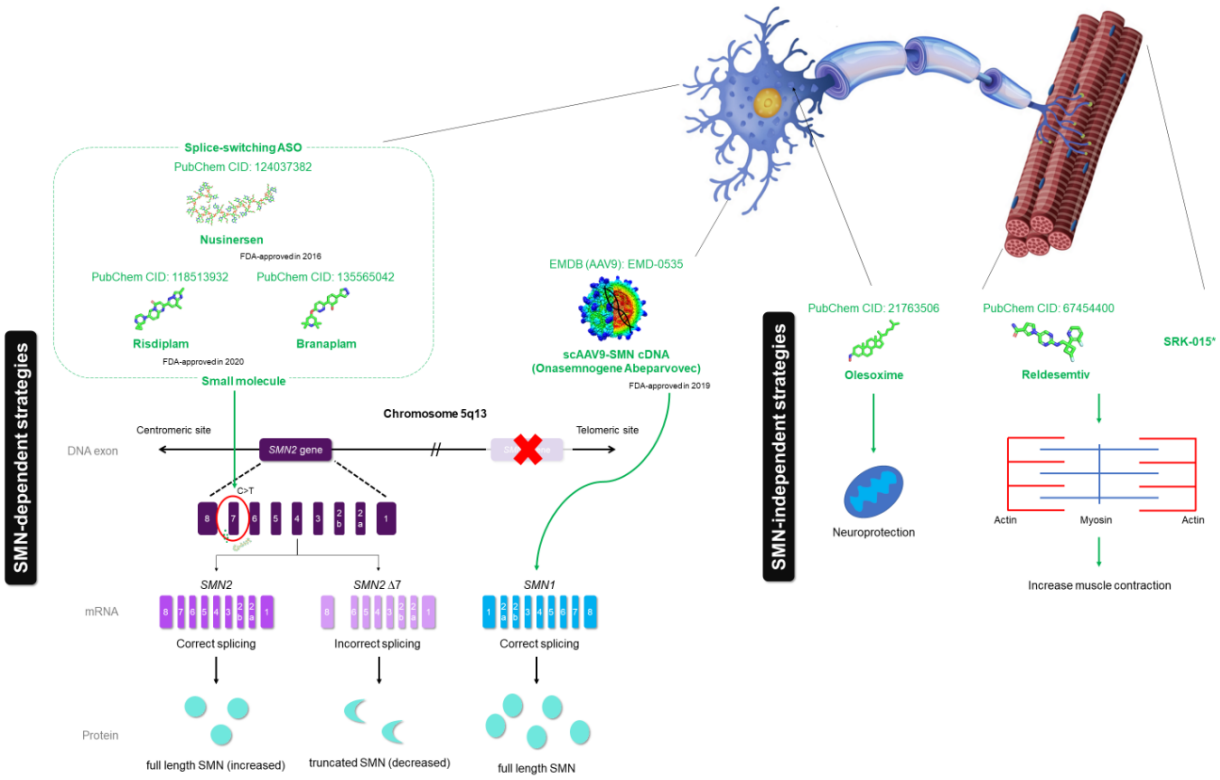

Figure 3. Therapeutic mechanism of SMA drugs, including three FDA-approved drugs (nusinersen, onasemnogene abeparvovec and the recent FDA-approved drug risdiplam) and drugs that are in clinical trials (branaplam, olesoxime, reldesemtiv and SRK-015). Nusinersen (PubChem CID: 124037382), a synthetic antisense oligonucleotide (ASO), is designed to hybridize intronic splicing silencer N1 (ISS-N1), which is heterogenous nuclear ribonucleoprotein (hnRNP) A1-dependent, to facilitate accurate splicing of *SMN2* transcripts. Onasemnogene abeparvovec (no available structure) is a gene therapy that targets the *SMN1* gene replacement using adenovirus vector AAV9 (EMDB: EMD-0535). Risdiplam (PubChem CID: 118513932) and branaplam (PubChem CID: 135565042) are small molecules that have the same mechanism of action as nusinersen. The red 'X' mark represents the deleted *SMN1* gene. Other than SMN-dependent drugs, olesoxime (PubChem CID: 21763506) acts as neuroprotective compound, while reldesemtiv (PubChem CID: 67454400) and SRK-015 act as a fast skeletal muscle troponin activator (FSTA) and myostatin inhibitor, respectively, to increase muscle contraction.

Despite the discovery of promising therapeutic strategies, the limitations, including the treatment viability (in the case of nusinersen), long-term effects, side effects and cost, among others, are highlighted. As the drugs need to pass through the blood–brain barrier (BBB), nusinersen must be administrated locally through an intrathecal injection. This route of administration is challenging and requires sophisticated personnel and technique, such as image-guided technique, particularly for patients with scoliosis and/or spinal deformity [56]. Moreover, elevated costs of nusinersen (~USD $125,000 per injection) associated with screening and subsequent treatment (~USD $750,000 in the first year and ~USD $375,000 annually for subsequent year) place this drug among the most expensive drugs [6][57]. For the latest approved gene therapy, onasemnogene abeparvovec costs ~USD $2.125 million per injection, although only a single treatment is required for each SMA type I patient [58], while the cost of risdiplam (the most recent FDA-approved drug) is yet unknown. Additionally, as all are relatively new therapies, there are no longitudinal studies for long-term effects, although there are a plethora of studies for side effects. Therefore, a more cost-effective drug with an alternative route of administration is required for this devastating SMA.

4. Computer-Aided Drug Design (CADD)—The Open Window of Therapeutic Agents

With technological advances in the areas of molecular structure characterization, computational science and molecular biology, CADD is a promising avenue to facilitate the discovery, design and optimization of potential therapeutic agents in the era of big data. Not only does it reduce the time for drug discovery, CADD plays a prominent role in reducing the quantity of testing molecules in vitro or in vivo [59][60]. By predicting the numerous small molecules, either natural or synthetic compounds, that bind favorably to the target macromolecules, the number of trial experiments can be minimized.

Of neurological disorders, the discovery of efficient CNS drugs is more challenging as compared with other diseases [61]. There are several challenges, in general, throughout the drug discovery process. The most notable obstacle in the process of lead optimization is the presence of the blood–brain barrier that restricts the flow of molecules to the brain. Nonetheless, it is possible to overcome and predict biological activity, pharmacokinetics (absorption, distribution, metabolism and extraction; ADME) as well as toxicity with the advent of more sophisticated computational approaches such as the high throughput screening (HTS) method and CNS multiparameter optimization algorithm [61][62].

Approved or investigated drugs, either SMN-dependent or SMN-independent, were identified with an impressive preclinical or clinical effect; however, none of them is able to cure the disease alone. Hence, this invokes the compelling motivation to implement a CADD approach to speed up the development of the SMA drug—*in silico* drug repurposing, network-driven drug discovery (NDD) and artificial intelligence (AI)-assisted drug discovery (AID).

4.1. In Silico Drug Repurposing

Drug repurposing, also known as drug repositioning, is one of the emerging potential approaches to circumvent the cost and time required for the development of an efficacious treatment [60][63]. It is defined as a process of identifying new therapeutic indications for an approved drug. Recently, with the encouragement of fast track marketing authorization procedure (FDA approvals), this approach has been widely used for rare diseases [63], including SMA [64], because it offers several benefits over the classical de novo development process of drugs. The approved drug compounds, in essence, have passed safety efficacy, allowing an omission of Phase I clinical trials [64][65].

Several studies have successfully repurposed FDA-approved drugs for SMA treatment and showed plausible in vitro activities, such as enhancing the SMN2 promoter activity, modulating SMN2 splicing and stabilizing *SMN2* mRNA or SMN protein [51][64][66]. Histone deacetylase inhibitors (HDAC), including sodium butyrate, phenylbutyrate and valproic acid (VPA), among others, to date, have been explored with SMN2 promoter activity [66][67][68][69][70]. In essence, SMN-independent drugs are centred on neuroprotective and muscle enhancing approaches. In referencing the localization of the SMN protein in neuronal cells, neuroprotective drugs for other CNS diseases could be a better option to reposition for preventing and/or delaying motor neuron death in SMA. Approved neuroprotective drugs, such as riluzole, hydroxyurea and rasagiline, which modulate regulatory pathways in CNS, may be an option for SMA therapy [51][64][66].

Given the potential of the drug repurposing approach, with the combination of publicly available databases and computational methods, the *in silico*-based approach may provide benefits, in terms of time and cost, towards the drug discovery process by narrowing down the top hits through *in silico* validations [71]. Public repositories for relevant experimental and biological data, including chemical structures, gene expression, drug disease association, phenotypic traits, side effects and more, are treasure troves for *in silico* drug repurposing. Owing to the wealth of multi-omics data, different methods have been adopted in drug repurposing, which can be divided into two major categories: (i) drug-oriented and (ii) disease/therapy-oriented [72].

Drug-oriented drug repurposing strategies require the knowledge of cheminformatics and bioinformatics as a foundation, including drug information, chemical structures of drug and target, drug-target network, signalling or metabolic pathway and genomic information. The disease-oriented approach is only applicable if the information of the disease model is available and commonly used to study the contribution of pharmacological characteristics towards drug repositioning effort on a particular disease. The blooming of drug repurposing resources and the advances in computational sciences give rise to the development of novel algorithms/tools and approaches that are capable of capitalizing on publicly available data.

4.2. Network-Driven Drug Discovery (NDD)

Network biology epitomizes the cell as a cluster of molecules interacting with one another and aims to illustrate the emergence of cellular phenotype from the network of molecular interactions [73]. The networks can be regarded as establishing the mechanistic bridge between the constituent molecules of a cell and the phenotypes that the cells demonstrate. This perspective alone considers the cellular mechanism of disease to be materialized due to networks of pathological interactions that occur only in the disease state. In this context, drug discovery can, hence, be perceived as the search for agents that significantly disrupt these pathological networks. NDD, as a whole, aims to identify signatures of molecular perturbations; that is, collections of multiple proteins, that significantly disturb the structural integrity of the cellular networks bringing forth the targeted disease mechanism [74]. The search space of therapeutics, such as small molecules, biologics or other agents, can then be screened and narrowed down based on their ability to produce the identified perturbation signature. It should be acknowledged that the compounds of this scheme are not expected to directly bind to all proteins within the identified signature, but rather to produce a downstream, functional effect on the

molecules making up the signature [75]. This approach is far removed from the traditional target-driven drug discovery that focuses on specific drug targets, whose downstream effects will significantly perturb the disease phenotype without much emphasis on cellular networks for understanding the underlying disease mechanisms.

As opposed to the canonical SMN-independent treatment based on many disease-modifying pathways, potential drug targets may be found on the periphery of the pathways using the NDD approach. A network analysis based on the two main proteins (**Figure 4**), SMN1 and SMN2, as protein input in GeneMANIA (https://genemania.org/) [76], has generated a network of putative interacting proteins that works in unison to bring about the phenotypes as seen in SMA. Proteins such as GEMINs [77], SNRPB [78], DDX20 [79] and PFN2 [80] appear to be highly correlated to the functioning of SMN1 and SMN2. These proteins are essential to SMN in forming macromolecular complexes (e.g., SMN-GEMINs, SMN-snRNPs) to chaperon the assembly of small nuclear ribonucleoproteins (snRNPs) that are vital to pre-mRNA splicing for producing the final SMN1 and SMN2 proteins [77]. Modulating these proteins in the cellular network within the context of SMA may serve as an opportunity to develop novel therapeutics complementary to the conventional SMN-dependent treatments in addressing the challenge of creating a robust and sustainable solution to curing SMA.

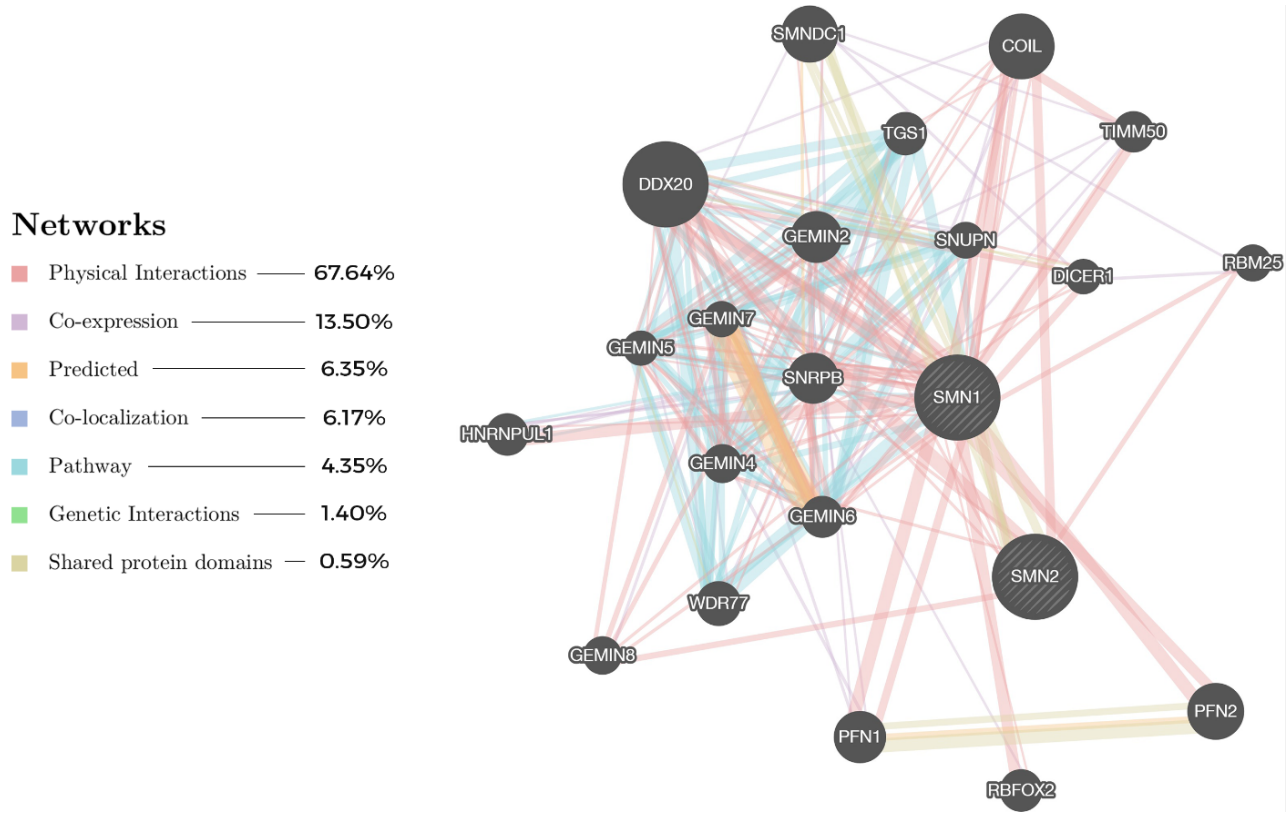

Figure 4. Protein network based on two main proteins, SMN1 and SMN2, and their respective interactions with other proteins related to SMA, generated using GeneMANIA [76] (https://genemania.org/). The most prevalent network relationship, reported by literature, among the proteins is physical interactions (pink color) at 67.64%, as visually shown by the line thickness, while the smallest belong to the shared protein domains at 0.59%.

With the advances of network biology, the rapid growth of publicly available biomedical data and advanced computational analytics, the NDD approach, a mechanistic based approach, proposes an alternative to identify the novel target as potential SMN-independent treatment.

4.3. AI-Assisted Drug Discovery (AID)

Driven by the big data in the field of biomedical and/or healthcare, the advancement of algorithms and technology such as deep learning (DL), graphical processing units (GPUs) and Google's tensor processing units (TPUs) enable better predictive capability by shortening the computing time [81][82]. To date, AI has been extensively adopted to support healthcare services and research. Virtual screening [83], quantitative structure-activity relationship (QSAR) [84], de novo drug design [85][86], drug repurposing [87] and chemical space visualization [88] utilized ML extensively to reduce the gap in the conventional methods in drug discovery, while DL shows promise in proposing potent drug candidates using their properties and toxicity risks [9]. Uptake from the pharmaceutical industry is still lagged, especially for rare diseases. Given the breadth of AID, we summarized the pipeline and its pre-requisites (Figure 5).

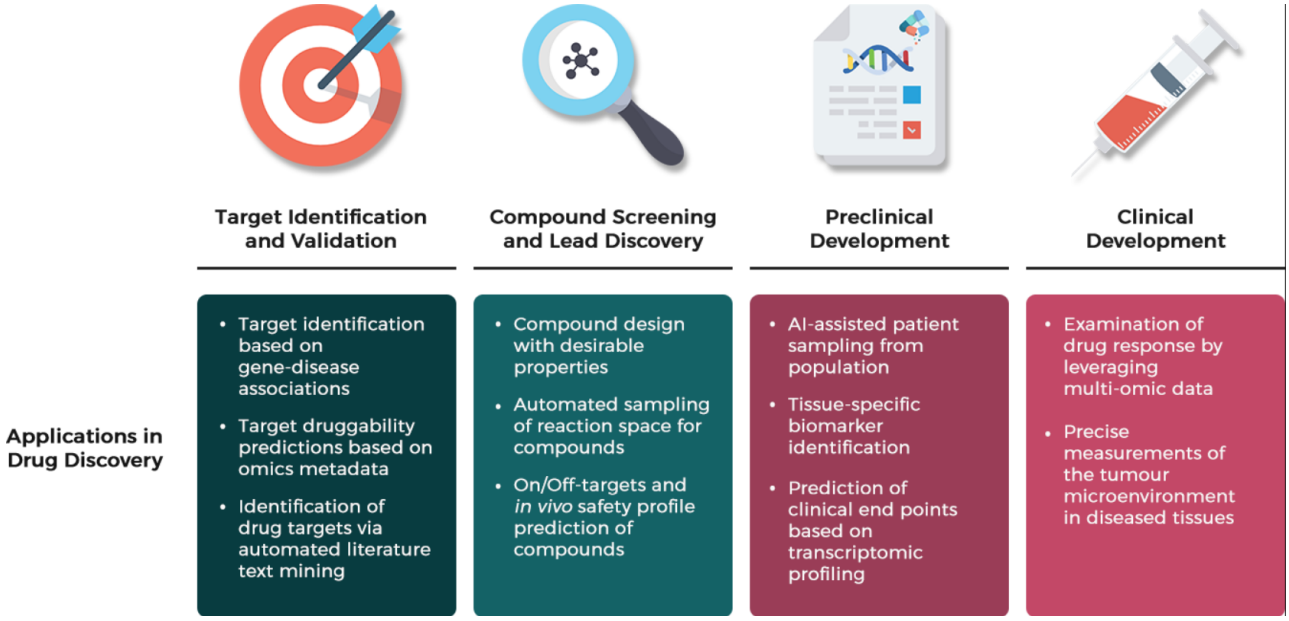

Figure 5. Machine learning applications in the drug discovery pipeline. Promising developments of pioneering ML research has brought forth unprecedented advances across various stages of the traditional drug development pipeline, especially the concept of automation in the early drug discovery process of target identification and validation & compound screening and lead discovery; relying on the domain of NLP in AI to find prospective drug targets by scanning upon thousands of relevant literature based on contextual information in research papers, and integrating AI with synthesis robots to explore unknown reaction space to search for drug candidates in which multiple chemical experiments are conducted automatically in real-time to assess the reproducibility of chemical reactions and discover new reaction outcomes. AI in the preclinical development has been a game-changer for patient selection in Phase II and III clinical trials by identifying and predicting human-relevant biomarkers of diseases, thus preventing unnecessary toxicities and side effects of consuming the experimental drugs for the designated patients [89].

Through a closer inspection of AI techniques in accelerating drug discovery, there are several common machine learning methods being employed to address the challenges in two major areas of drug development: (i) design and discovery and preclinical research; and (ii) clinical research and safety monitoring.

5. Conclusions

The task of finding a successful, novel drug as treatment for common diseases is predominantly a daunting yet arduous process, which is even more challenging for a rare genetic neurological disorder such as SMA. Many research and development pharmaceutical companies and research institutions are hesitant to pursue the drug development for rare diseases due to the small market size, high cost, possibly low return and lack of information about the disease, drugs and corresponding drug targets. Recently, CADD approaches have shown promising potentials in facilitating the drug discovery process and may be able to overcome the limiting bottlenecks of its traditional counterparts. Along with the advances of the knowledge of computational biology and informatics database, the opportunities provided by drug repurposing cannot be underestimated. The interactions of a drug and a target is a critical point of drug discovery. This information aids to establish correlations between diseases and targets in order to determine the therapeutic effect of drugs on various diseases. Hence, the well-known drug–disease relationships that has been established using network biology will help accelerate the target identification and lead optimization process for pre-clinical drug development. Integrated with the domain-specific AI in the ‘chemical big data’, the novel approach could potentially serve as a panacea by increasing the efficiency of certain aspects of the drug discovery process. Despite the promising potential offered by CADD, there are several challenges, including the access of databases consisting all the approved drugs and their detailed profiles, in-depth knowledge of disease, particularly for multifaceted disease, among others to capitalize the benefit of CADD in advancing the domain of drug research and development.

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