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New approaches for challenging therapeutic targets

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Abstract

Despite successes with new drug approvals over the past two decades through conventional drug development approaches, many human diseases remain intractable to current therapeutic interventions. Possible barriers may be that the complexity of the target, and disease biology, are impervious to such conventional drug development approaches. The US National Institutes of Health hosted a workshop with the goal of identifying challenges and opportunities with alternative modalities for developing treatments across diseases associated with historically undruggable targets. This report highlights key issues discussed during the workshop that, if addressed, could expand the pool of therapeutic approaches for treating various diseases.

Teaser

Despite successes with new drug approvals, many human diseases remain intractable to conventional therapeutic interventions. This report highlights areas to expand the pool of therapeutic approaches for treating disease discussed at a recent NIH workshop.

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Declarations of interest

The authors declare no conflicts of interest and have nothing to disclose.

Disclaimer

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intractable disease; alternative modalities; undruggable targets

Introduction

Conventional target-based drug discovery projects that utilize small molecules (i.e. chemically synthesized entities) rely heavily on *in vitro* assay screens to identify compounds that may bind to specific targets, such as enzymes or receptors (see Table 1 for definitions of major terms used within this work). This approach has provided the foundation for early stage target-based drug discovery with small molecules, where those identified 'hit' compounds may then be optimized through medicinal chemistry into compounds with improved target binding and druglike properties (i.e. 'leads') for further evaluation, and ultimately into possible clinical development candidates. However, significant numbers of human diseases are caused by currently undruggable proteins or mechanisms, meaning that they cannot readily be treated via current conventional target-based approaches using small molecules.[1,2] This is often reflected in the inability to identify putative binding locations at the active site (or identify hidden active sites) needed to modulate disease biology. It has been estimated that approximately 10% of the genes in the human genome code for a protein that may be considered druggable by small molecules having druglike properties, with up to ~5% being both druggable and disease relevant.[3] As recently as 2017, only four protein classes (GPCRs, kinases, ion channels, and nuclear receptors) have been identified as being responsible for the therapeutic effect of 70% of approved small-molecule drugs, and efforts have been made through computational methods to deprioritize targets that are not worth pursuing due to druggability concerns.[4,5] It is no surprise then that entire classes of diseases are seldom treated by conventional small-molecule approaches. Examples include diseases caused by protein aggregation or protein–protein interaction (PPI; as seen in neurodegenerative and prion diseases), diseases caused by proteins without a clear binding pocket (as seen in some cancers and other diseases), and diseases caused by something other than a single protein within a limited number of protein families.[4,6,7] Accordingly, relying solely on conventional small-molecule approaches neglects the needs of a large number of patients spanning multiple disease areas.

The need for innovative treatment modalities to attack intractable diseases is not unique to any single disease field, and it is expected that many disease areas could benefit from the development of novel treatment modalities. A prominent example in recent years has been the development of proximity-based protein degradation methods, such as proteolysis-targeting chimeras (PROTACS), and examples of candidate therapeutics are being clinically evaluated for various cancers.[8,9] The emergence of molecular degraders and other new drug development technologies, along with the identification of an ever-increasing number of novel molecular targets implicated in human disease, has primed the field to pursue the formidable task of tackling challenging drug targets. To identify key areas for improvement or new discovery, the National Institutes of Health's (NIH) National Center for Advancing Translational Sciences (NCATS), cohosted a workshop titled 'The Quest for Innovative Molecular Treatment Modalities for Intractable Disease Targets' with the National Cancer

Institute (NCI), the National Institute of Neurological Disorders and Stroke (NINDS), and the National Institute of Allergy and Infectious Diseases (NIAID) on November 17–18, 2022. The goal of this workshop was to identify opportunities for developing treatments against targets historically considered undruggable via current methods of small-molecule-based drug discovery.[10] This workshop focused on identifying (i) protein families, molecular entities, and disease areas most in need of innovative therapeutic modalities, (ii) new or emerging technologies that could be leveraged to address limitations of current treatment modalities, and (iii) gaps or pressure points preventing or slowing the development of strategies for treating human diseases associated with undruggable targets. Session topics were identified based on interviews with subject matter experts representing industry, academia, and government. Topics included a focus on disease areas where novel modalities are (or could be) applied to new targets, new and emerging methods for disease treatment, and advances in drug delivery technologies. It should be recognized that the topics covered in this workshop were not intended to be ‘all-inclusive’ with respect to new approaches for challenging targets, but rather were topics deemed of priority for discussion by the session leaders in the limited time available. While there are additional emerging approaches and technologies (e.g. covalent inhibitors, allosteric modulators, and DNA-encoded libraries) that each play roles as well, these were not among the prioritized topics.

Workshop attendees representing a diversity of backgrounds, expertise, career stages (i.e. senior leaders, midcareer, junior, trainees) and institutions engaged in discussions around the various workshop topics. The following sections report on the key issues and challenges identified in each topic area, including potential alternative therapeutic modalities that fall outside of conventional small-molecule drug discovery efforts that may be utilized for the treatment of human diseases associated with undruggable targets (Table 2).

Targets beyond conventionally druggable proteins

Recent advances in drug discovery technologies and our understanding of disease biology have expanded the scope of potential drug targets beyond proteins that are historically considered ‘druggable’. It is now possible to identify new targetable pockets on proteins previously believed to be undruggable if a protein is observed under new conditions, such as when it is mutated, as was demonstrated with KRAS, or when the protein binds to another protein causing exposure of a cryptic site.[11,12] We have also learned a significant amount about non-protein targets, such as RNA, organelles, and lipids, including their role in human disease. The sessions held during this segment of the workshop delved more deeply into these target areas and how both conventional and novel methods could be utilized to treat them.

Session 1: RNA

It is estimated that only ~1.5% of the human genome encodes ~21,000 distinct protein-coding genes and that most of the genome is transcribed into RNA.[13] Recent advances have indicated that RNA can be leveraged as a target to treat diseases not only caused as a direct result of RNA dysfunction but also diseases in which an undruggable protein is causative.[14] RNA has both low and high structure regions, so there are two primary ways to affect RNA:

(i) using small molecules that can target regions of RNA that have stable structure, and (ii) using antisense oligonucleotides (ASOs) that block function or degrade RNAs that are composed of regions with significant structural variability.[14,15] RNA can also be targeted in several forms:

- Transfer RNA (tRNA) and messenger RNA (mRNA): tRNAs can become dysregulated in many diseases, including several neurological disorders and rare diseases, causing disruption of the translation of mRNA into a protein.[16] The targeting of tRNA, and even utilizing tRNA itself as a therapy, has been suggested as a potentially novel method for treating human disease.[17]
- PIWI protein-binding RNAs (piRNAs): piRNAs are also targetable and have some advantages over other RNAs; they have an O-CH3 3' end modification that prevents them from being degraded as readily as the other RNA types. They are also dicer independent, making off-target effects very minimal, and they have more regulatory functions than other RNAs, including within the pretranscriptional, transcriptional, posttranscriptional, and translational steps.[18]
- Pre-mRNA and the spliceosome: the spliceosome functions as a catalyst for the removal of introns from nuclear pre-mRNA. Spliceosome modifications could reveal new ways to treat intractable diseases, such as Huntington's disease.[19]
- Ribosomes: ribosomes are primary protein synthesis machines and have been implicated in a variety of diseases including bacterial infection and cancer.[20] Unlike other RNAs where the effort has been focused on targeting secondary structure elements, the ribosome is unique in that it presents many vast and highly expansive targetable binding pockets that are inviting sites for small molecules.[20]

In addition, the study of RNAs as targets can provide an opportunity to further understand off-target effects of drugs as many protein-targeted medicines may also be targeting RNAs, causing significant off-target effects. Ultimately, RNAs are an important emerging target class for the development of therapeutics, and progress in the development of small-molecule RNA targeting agents, such as ribonuclease-targeting chimeras (e.g. RIBOTACs), should provide exciting future possibilities.[14,15]

Session 2: Protein Stability

Errors in protein folding underlie numerous diseases, including for example type 2 diabetes, cataracts, Alzheimer's disease, and Huntington's disease, and there are a number of ways that these folding errors can occur.[21-23] In general, loss of function/abnormal trafficking of proteins and protein misfolding represent two major types of aberrant protein stability events associated with disease. For example, a single point mutation in the beta-globin gene in sickle cell anemia leads to the production of an abnormal version of a subunit of hemoglobin, resulting in improper folding and loss of function of the protein responsible for carrying oxygen in red blood cells.[21] Abnormal trafficking due to protein misfolding can be seen in diseases such as cystic fibrosis (CF), in which protein misfolding causes rapid intracellular degradation of the CFTR protein, resulting in a reduced ability of the cell to shuttle chloride across the plasma membrane.[24] Protein aggregation and self-propagating

amyloids have been shown to be leading contributors to a number of diseases, including Parkinson's disease and prion diseases.[25] In many neurological diseases, misfolded proteins build up within cells or create amyloid plaques in the space between nerve cells, disrupting neuronal function. In the case of prion diseases, misfolding of either/both the wild-type and mutant forms of the prion protein can form a variety of specific misfolded conformers, resulting in self-propagating, pathogenic prion collections, each of which can cause its own pattern of disease.[26]

There are very few treatment options available for diseases caused by disruptions in protein stability, and it is believed that drug combinations might be one of the best options for the design of treatments moving forward. This is because some dysfunctional proteins need stabilization at several distal locations to restore function, as is the case with cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel mutations.[24] Trikafta is a notable example of this approach, featuring a combination of three drugs where a potentiator is combined with a protein fold corrector as well as a dual-function modulator, which serves to restore function in patients who have the F508del mutation leading to CF.[27] In the case of neuropathologic protein aggregates, there is a need to target several forms of misfolded protein at once. In the prion field, a new perspective that has arisen is to explore the stabilization of native prion proteins instead of targeting misfolded proteins, to prevent the formation of additional misfolded proteins.[28] Alternative approaches may include targeting mechanisms and factors that modulate pathologic misfolding of proteins *in vivo*, such as increased protein concentrations, post-translational modifications, PPIs, crosslinking, and/or oxidative stress/mitochondrial inhibitors.

In addition to well-defined three-dimensional proteins, a large fraction of the human proteome comprises proteins that, under physiological conditions, lack such well-ordered structure. These intrinsically disordered proteins (IDPs) exist as heterogenous ensembles of unstructured or partially structured configurations.[29] Some IDPs are involved in liquid–liquid phase separation (LLPS), which can result in membrane-less liquid droplets that, within a cell, can compartmentalize biomolecules like RNA-binding proteins. However, aberrant phase separation can result in the formation of fibrillar protein aggregates, such as are seen in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).[30,31] It is inherently difficult to develop treatments against these proteins as the standard pipeline of drug discovery starts with a well-recognized, well-characterized target with known structures, characteristics that IDPs do not have. To help overcome this challenge, recent advancements in molecular simulations have been found to be very useful for identifying druggable components of IDPs.[32]

Session 3: Other Novel Targets

Other non-protein targets, such as lipids, carbohydrates, organelles, and other complex cellular structures, offer exploratory therapeutic opportunities and were the focus of this workshop session.

Lipid rafts are cholesterol-rich, dynamic assemblies of proteins (i.e. receptors/channels) that float within cellular membranes. Specific 'inflammarafts' expressing Toll-like receptor 4 (TLR4), for example, are present on microglia and dorsal root ganglion (DRG) macrophages

and nociceptors.[33] As with nerve injury and inflammation, these TLR4 rafts are activated, leading to enlarged, stabilized rafts and aggregation of proexcitatory channels and receptors. The rafts serve as organizing platforms to initiate and sustain enhanced proinflammatory signaling. These effects can be reversed by targeting TLR4 raft cholesterol efflux using molecules such as those affecting apoA1 binding protein (A1BP), inhibition of ganglioside synthases, or control of sphingomyelinases. These approaches address raft-associated dysfunction, as occurs in many inflammatory pathologies, including pain, and represent investigational opportunities for tackling lipid raft-associated diseases.[33]

A variety of diseases, including many rare diseases, can result from malfunction of subcellular organelles. Disruption of the Golgi apparatus has been implicated in several diseases, including Alzheimer's disease, and causes significant downstream aberrant effects.[34] Likewise, mitochondrial disorders are characterized by multisystemic, complex, and variable clinical pathologies.[35] Diseases caused by organelle dysfunction or loss of function represent ripe areas for investigation, as treatment options remain very limited due to the complexity of treating these types of disorders. An emerging approach is the use of organelle-specific nanoplatforms, based on unique organelle characteristics, to achieve targeted treatments.[36]

Cellular structural components can contribute to disease pathology including several neuropathies, myopathies, skin disorders, liver diseases, metabolic dysfunctions, and premature aging syndromes, among others. Intermediate filaments (IFs) are cytoskeletal and nucleoskeletal structures that provide mechanical and stress-coping resilience to cells, contribute to subcellular and tissue-specific biological functions, and facilitate intracellular communication.[37] As significant disease pathology is caused by the functional dysregulation of the IF cytoskeleton through altered post-translational modifications, there is significant opportunity to explore these mechanisms for future treatments. Due to the wide range of diseases that IFs cause or contribute to, discovery of new treatments for IF pathologies would be a major leap forward in addressing a key patient population with significant unmet needs.

Alternative modalities: moving beyond conventional modalities

The failure of a small-molecule drug candidate to advance to the clinic can be attributed to several factors, including the challenges associated with the biological target as well as the properties of the molecule itself. As much of biology is regulated by PPIs where the interface between one protein and another is very flat, it is challenging for conventional reversibly binding small molecules to bind or disrupt such interactions. Targeted protein degraders (TPDs) have expanded the scope of small-molecule drug discovery beyond occupancy-based pharmacology and provide an opportunity to repurpose existing molecules as well as pursue targets that were once considered undruggable. Fragment-based drug design coupled with protein interaction stabilization techniques have shown promise in addressing such PPIs.[38] In this portion of the workshop, focus was placed on identifying alternative uses to existing modalities, highlighting emerging novel modalities, as well as discussing new drug delivery technologies.

Session 4: Repurposing Modalities

The development of TPDs represents an evolving therapeutic approach. Notable progress has been made in the design and use of PROTACs, bifunctional molecules that regulate protein function by forming a ternary complex with the target protein and an E3 ligase, degrading the target protein instead of inhibiting it and thereby providing more sensitivity to drug-resistant targets and a greater chance to affect nonenzymatic functions of a protein.[39-41] In 2019, PROTACs began entering the human therapeutics arena with the development and entry of two heterobifunctional degraders into first-in-human trials (ARV-110 and ARV-471).[8,9] These compounds have helped usher in the development of therapeutics based on protein degradation. Importantly, PROTACs have not only opened new opportunities for targeting but are also allowing researchers to return to 'older' targets (and molecules) where agents that modulated a protein's activity through occupancy-based pharmacology may not have been successful. As this field continues to expand, additional targeted degrader-based approaches have emerged, and protein degradation approaches are not just being considered after target occupancy-based approaches fail or when it might be a last resort, but also as a primary approach and in situations where degradation of the protein may be advantageous (e.g. overcoming protein aggregation).[42,43]

Molecular glues work based on the same underlying mechanism as PROTACs, i.e. the ubiquitination of a protein of interest with the help of an E3 ligase or, in the case of bacterial PROTACs, utilizing caseinolytic protease proteolytic subunit (ClpC) or other bacterial proteases for protein degradation, but unlike PROTACs they do not require a ligand binding site.[44,45] These degraders work by converting the target protein into a 'neosubstrate' for an E3 ligase by inducing a conformational change in the protein.[46] Molecular glues are increasingly being utilized to treat targets historically considered undruggable, and mechanisms for molecular glues have been shown to be diverse and broadly applicable for therapeutic targeting. However, most of these mechanisms have been based on serendipitous discoveries, so there is a need for a systematic effort to establish novel glue systems that can be leveraged against neosubstrates of high therapeutic relevance, particularly at the level of higher technical complexity. Continued efforts toward gaining an improved structural understanding of glue mechanisms can help address this challenge.[47]

While targeted protein degradation through PROTACs and molecular glues work by targeting specific proteins for ubiquitination and proteasomal degradation, there are other classes of disease-affiliated targets associated with faulty or overactive ubiquitination and degradation. These include many tumor suppressors in cancer as well as mutated and misfolded proteins, as is seen in CF; for these diseases, deubiquitinase-targeted chimeras (DUBTACs) have demonstrated utility in preclinical studies.[48] DUBTACs are heterobifunctional small molecules consisting of a deubiquitinase recruiter linked to a protein-targeting ligand, and they function by stabilizing the levels of proteins and preventing their degradation in a ubiquitin-dependent manner. While this technology has many potential applications, it is still in the early stages of development, and there is still a major need to design assays that will allow for rapid validation of proteins that can be stabilized by DUBTACs. Finally, the development of RIBOTACs, heterobifunctional small molecules that include a deubiquitinase recruiter and an RNA-targeting ligand (versus

protein targeting), represents another expansion of degrader-based technologies and provides an approach to directly affect RNA biology. The development of RIBOTACs is in its early stages and, as stated earlier, confers an exciting opportunity for directly targeting RNA with a small molecule.[14,15,42]

Session 5: Novel Modalities

While targeted degraders have expanded the druggable pool, the field of drug discovery still has a significant need for novel modalities to treat disease. It was highlighted that part of this need could be alleviated by having an expanded selection of drug target candidates, such as those that modulate post-translational processing. However, in these situations, there is an even greater need for an intimate understanding of the molecular biology of the disease state. This is highlighted in recent work in which preclinical models that capture dynamics within and crosstalk between relevant tumor compartments have led to the development of better oncology treatments.[49]

In addition to harnessing the ubiquitin pathway to promote controlled degradation of specific proteins, other chemical signals have been targeted to develop novel modalities and were discussed. These included post-translational modifications such as induced phosphorylation, acetylation, and of particular interest in this workshop, glycosylation of specific protein targets. Protein glycosylation was highlighted as it has a major role in numerous biological processes, and when glycosylation fails it can result in many diseases, including diseases of the immune response, cancer, neurodegeneration, and diabetes, among others.[50] Recent work has uncovered the benefits of targeting O-linked ß-*N*-acetyl glucosamine (O-GlcNAc), a post-translational modification found on thousands of nuclear, cytosolic, and mitochondrial proteins.[50] When this modification is targeted in breast cancer, it can potentiate inhibitory effects of breast cancer cells, thus providing an example of how these types of mechanisms can be exploited to modulate disease state.[51] This, and other post-translational modifications, suggest opportunities to expand the target landscape.

In addition to targeting post-translational modifications, macrocyclic molecules with their generally higher molecular weights and constrained geometries offer the opportunity to rationally engage challenging targets, such as PPIs.[52] To date, several macrocyclic drugs have been developed for various indications, including infectious diseases, cancers, Cushing's disease, anemia, and others.[53] Another approach for targeting PPIs may be through the use of miniproteins, which are small proteins (roughly <10 kDa in size) that are often densely cystine knotted with stable tertiary structure. Moreover, due to their smaller size, they may have greater tissue penetration than larger protein drugs.[54] An inspiring source of miniproteins is natural venom proteins, which have distinct druglike properties.[54]

While the above examples show how the field could expand outside of the conventional small-molecule drug discovery pathway, several challenges have been identified. These include underdeveloped basic discovery platforms, a misalignment of features that constitute 'druglikeness', and a lack of clear safety assessment criteria for preclinical drug development. Finally, incorporation of both forward and reverse translation in drug development, with forward translation being the process of implementing basic research discoveries into practice and reverse translation being the process of elucidating the

mechanistic basis of clinical observations, represents a gap to fill for enabling the development of novel modalities.[55]

Session 6: Novel Drug Delivery

In addition to the development of novel drugs, it is important to address drug delivery, which can be a contributing factor to drug failure in clinical trials.[56] The most commonly utilized methods of drug administration are oral and intravenous (IV) dosing, which can yield high systemic exposure but are also prone to nonspecific biodistribution and, consequently, toxicities. The inability to achieve effectual drug concentrations specifically at the site of action at a therapeutically acceptable dose and suppress off-target effects resulting from nonspecific tissue distribution following systemic administration are pervasive challenges in the field. The development of novel drug delivery approaches has been an active area of investigation to overcome these challenges, and the technologies that have gained prominence include (but are not limited to) nanoparticle-based delivery systems, liposomes and lipid-based carriers, micelles, antibody–drug conjugates, pH-responsive capsules, swellable hydrogels, and transdermal patches. However, despite the progress in the field of drug delivery systems, these methods may not address specificity for a particular organ or tissue site; this was a topic of discussion in this session, along with emerging technologies. For drug delivery directed to the central nervous system (CNS), which is notoriously hard to target due to the blood–brain barrier, two interesting endogenous approaches were highlighted at this workshop. One approach described extracellular vesicles (EVs) that penetrate the CNS from the periphery and exit the CNS via the circulation, cerebrospinal fluid, or lymphatic system, allowing them to be exploited as either biomarkers or therapeutic carriers.[57] Also described were how endogenously produced EVs can be engineered to enhance targeting and be developed for more advanced loading systems to facilitate encapsulation of therapeutic targets for delivery to the CNS.[57] Another example described for harnessing the body's own systems to treat neurological diseases included supplementing the gut microbiome with a genetically engineered, orally delivered *E.coli* that is able to endogenously produce and deliver to the CNS beneficial levels of L-DOPA. Results from early studies in mouse models of Parkinson's disease have shown improvements in motor, cognitive and mood-related tasks in mice orally supplemented in this way.[58]

Ex vivo autologous stem cell gene therapy, in which a patient's own stem cells are harvested, cultured, re-engineered and then reinfused back into a patient, has shown clinical utility for treatment of hematological disorders; however, this is a highly intensive protocol that may result in treatment-associated toxicities and significant patient distress. To address this, a new approach was introduced in which antibody-targeted lipid nanoparticles (LNPs) are used to directly deliver RNA [small interfering RNA (siRNA) or mRNA] to hematopoietic stem cells (HSCs). Recently, this approach has been successfully demonstrated by delivering RNA to hematopoietic HSCs in rodents utilizing a c-kit (CD117) antibody-targeted LNP.[59] In another new cell therapy approach, mesenchymal stem cells (MSCs) can be engineered with targeting leucine zippers that aid in the formation of a living therapeutic cell depot *in vivo*. In animal studies, bone-derived MSCs that are surface decorated with heterodimerizing leucine zippers, termed ZipperCells, have been demonstrated to form targeted cell depots at sites of damage caused by myocardial infarction.[60]

Photoinduced drug release is an emerging field of drug delivery with many new approaches still being developed. The use of light-responsive carriers, such as liposomes, can be finely tuned based on the physical properties of the carrier and payload, as well as the irradiating wavelengths, to deliver drugs to the relevant tissue. Such an approach was described during the workshop and can be implemented through several techniques, including modification of the hydrophobicity of a nanocarrier constituent to make it more soluble, introducing local defects within a nanocarrier to make it more porous, or concentrating heat for thermosensitive nanocarriers to release their cargo. These methods offer the promise of exquisite control over timing and *in vivo* release of payloaded drugs.[61]

Concluding remarks

As our understanding of disease biology and technology continues to advance, alternative treatment modalities are likely to play an increasingly important role in drug development and patient care. This workshop brought to the forefront various novel targets for treatment outside of the proteins most often targeted by conventional therapeutic methods. During the course of discussions around these targets, several key points were highlighted and should be considered when identifying novel targets in disease:

- Protein classes that have been considered undruggable may be druggable, just not in the conventional sense; it is possible to identify new targetable pockets on difficult -to-drug proteins if the protein is examined under new conditions or targeted via an alternative pathway, such as degradation.
- Intrinsic disorder in a target is not an insurmountable barrier to drug development, especially with advances in molecular simulations, development of protein degraders, and increasing understanding of the structure–function relationship of macromolecules.
- There is a need for an intimate understanding of diseases at the molecular level and for a greater understanding of the contribution of multiple targets to some diseases. Some diseases will require combination treatment approaches, which presents both a challenge and an opportunity for new therapeutic approaches.
- While there are many protein targets available for developing targeted therapeutics, it is becoming increasingly evident that many diseases have non-protein or complex components contributing to disease state, which in the future could be considered for targeted treatment.

In addition to discussions around targets, the workshop aimed to highlight new and emerging modalities that could benefit the field of drug discovery. To fully realize the potential of new and emerging treatment modalities, several key points that emerged from the workshop should be considered when thinking about novel methods:

- As the field expands into even more challenging targets, the modalities will similarly become more complex. These modalities may include new therapeutic approaches that attack multiple targets with a single covalently designed molecule or bifunctional molecule.

- Target degradation-based approaches are evolving and are anticipated to increasingly be utilized in drug discovery.
- Despite improvements in knowledge of targets and modalities, the development of delivery approaches that enable enhanced delivery of therapeutics to the site of action remains an area of need.

Along with a deeper understanding of disease mechanisms, the range of drug targets is likely to grow even further. The continued development of innovative technologies in combination with an expanding toolbox of targeting approaches has the potential to overcome many of the limitations of conventional therapeutic interventions, and this may lead to the development of more effective treatments.

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Highlights:

- Many human diseases remain intractable to conventional therapeutic interventions.
- There is a need for innovative treatment modalities to treat these diseases.
- This report highlights key issues discussed at a workshop on novel modalities.
- Novel approaches are emerging to treat previously undruggable targets in disease.

TABLE 1

Commonly used terms in this paper

Term	Definition
Conventional drug discovery	Drug discovery efforts using small molecules (typically up to 500 Daltons, produced through fully synthetic or semisynthetic methods), which reversibly bind to a target as either an inhibitor, activator, agonist, or antagonist, to achieve a level of target occupancy that elicits an effect on the biology associated with the bound target
Undruggable Targets	Targets that cannot be engaged with high affinity through ‘conventional’ means
Intractable Disease	A disease that is not easily treated, relieved, or cured, or is unable to be treated, relieved, or cured through currently available therapies
Alternative Modalities	The universe of therapeutic approaches that does not fit within the definition of ‘conventional’

TABLE 2

Summary of workshop discussion highlights: The Quest for Innovative Molecular Treatment Modalities for Intractable Disease Targets

Session	Key Point	Challenges	Opportunities	References
RNA	RNA can be leveraged to treat not only diseases caused as a direct result of RNA dysfunction but also diseases in which perturbation is caused by a difficult-to-drug protein	• RNAs have low and high structure regions, making them highly dynamic and hard to target • RNA can be relatively unstable/transient, affecting efficacy of RNA-targeted treatments	• RNA can be targeted in several forms (tRNA, piRNA, mRNA, etc.) • Small-molecule agents targeting RNA can have multiple effects including targeted degradation of RNA • Utilization of RNA can inform off-target effects of drugs	13–20
Protein Stability	It is estimated that approximately half of human disease is caused by protein misfolding or defects in protein stability	• Many proteins, under normal physiological conditions, lack ordered 3D structures (e.g. intrinsically disordered proteins) • Some misfolded proteins (e.g. prions) can form ‘strains’ with resistance to therapies	Landmark successes with treatments that refold or stabilize protein conformations to recapitulate an effector function have pioneered a new way of thinking about drug development • Combinatorial complexity: some protein misfolds may require combinations of agents, and some agents may target more than one misfolded protein • Molecular simulations are advancing to a level that they may be applied to intrinsically disordered proteins	21–32
Other Novel Targets	The concept of what a target can be is expanding to include other cellular entities such as organelles and non-protein targets	• Organelles are highly complex and do not fit within the traditional concept of a target • Small-molecule drug discovery techniques have been mostly protein oriented	Cellular surfaces feature significant non-protein composition and potentially offer fertile ground for discovery of new nonconventional drug targets • Other nonconventional targets that are emerging include lipid rafts, Golgi apparati, mitochondria, and cytoskeletal features like intermediate filaments • Emerging nanotechnology methods can help identify target-specific organelles	33–37
Repurposing Modalities	Repurposing of existing drugs or of advanced investigational agents for new indications represents a continually growing resource to mine.	• Conventional modalities have been tailored to a target, reducing the ability to move across target categories • The existing pharmacopeia represents a conventional drug discovery bias in its generation, and it is not clear if this bias will limit the discovery of new modalities (but new modalities will add to and evolve this resource)	• Repurposing of the cell’s own machinery to address targets previously thought to be undruggable • Slight modifications to more established systems can yield significant new modalities to treat diseases	8,9,14,15,39–47
Novel Modalities	Targeted protein degradation and novel modalities have expanded the tools available to medicinal chemists	• Redefining our understanding of druglike physical properties and criteria for candidate selection • Retooling of discovery platforms to address discovery of novel modalities • Need for safety assessment criteria for new modalities	Utilization of heterobifunctional molecules (e.g. PROTACs, DUBTACs, RIBOTACs molecular glues, and many other variations) • Utilization of miniproteins, macrocycles and other alternatives to conventional small molecules to treat disease • De-risking and expanding the envelope of use of covalently modifying agents as potential new therapeutics	49–55
Novel Drug Delivery	There has been tremendous progress in the development of drug delivery systems over the last decade applicable to targeting undruggable targets	• Many *In vivo* compartments, like the blood-brain barrier (BBB), which blocks the passage of more than 98% of small-molecule drugs, continue to present pharmacokinetic (PK) challenges • Correct timing and release of a drug from its delivery system is another layer of complexity in the overall PK of a new therapeutic • Drug delivery systems can involve sophisticated technologies and/or expertise, which may not be easily accessible to academic researchers	• Tissue specificity can be enhanced with application of nanoparticle formulations, bioengineered microbiome-based systems, and nonviral DNA and genome editing systems • PK distribution of drugs may be achieved using extracellular vesicles or bioengineering of the body’s own biological systems • The application of photoactivated drug targeting is expanding in new directions	56–61