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Bachelor's thesis literature review

Autophagy-Mediated Targeted Protein Degradation: Mechanisms, Therapeutic Potential, and Future Directions

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Abstract:

Over the last decades, targeted protein degradation (TPD) has gained attention as a promising therapeutic strategy. However, proteasome-dependent technologies like PROTACs are largely restricted to soluble proteins within the cytosol. In contrast, autophagy-mediated TPD technologies, such as AUTOTACs, AUTACs, ATTECs and LYTACs expand the degradable targets to include aggregated proteins, organelles and membrane or extracellular proteins. This review aims to offer a comparative analysis of lysosome-targeting strategies, highlighting their distinct mechanisms of action. These include p62–mediated clearance of protein aggregates (AUTOTACs), K63 ubiquitin tagging (AUTACs), ATG8-based cargo tethering (ATTECs) and receptor-mediated lysosomal trafficking (LYTACs). These technologies are showing promising results in degrading disease-relevant substrates such as α-synuclein, mutant tau, huntingtin, lipid droplets, and PD-L1 in preclinical models.

Despite the promising potential of these degraders, several major challenges remain. Many exhibit poor solubility, limited cellular or BBB permeability and often rely on stoichiometric action rather than catalytic action. Additionally, receptor recycling bottlenecks (e.g., in LYTACs) and impaired autophagy mechanisms in target diseases (e.g., in Parkinson's Disease) may reduce efficacy. Delivery innovations such as nanocarriers and receptor-mediated transcytosis are actively being explored to overcome these barriers.

Altogether, autophagy-mediated TPD strategies offer a powerful addition to the degradation toolbox. Strategic advances in drug design, delivery, and receptor targeting will be essential to unlock their full therapeutic potential within oncology, neurodegeneration, and beyond.

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Introduction:

Despite the widespread use of small-molecule inhibitors in drug discovery, their reliance on binding to active sites renders many disease-relevant proteins undruggable (P. Wu et al., 2015). As a result, the controlled degradation of disease-causing proteins through targeted protein degradation (TPD) has emerged as a promising therapeutic strategy for the treatment of various diseases, including certain types of cancer, neurodegenerative diseases (NDs), and even genetic disorders such as Down syndrome (DS) (Akwa et al., 2022; Tang et al., 2023; Tan et al., 2023). During the early 2000s, Sakamoto et al. (2001) developed the first proteolysis targeting chimera (PROTAC), a chimeric molecule that catalyzes the degradation of a protein of interest (POI) through linking it with an E3 ubiquitin ligase that ubiquitinates the POI. Effectively marking the POI to be degraded through the ubiquitination-proteasome system (UPS). The field of PROTACs has been developing rapidly, with several molecules already in clinical trials (Chen et al., 2024; Hurvitz et al., 2023; *Summary of PROTAC Degraders in Clinical Trials | Biopharma PEG*, n.d.). Furthermore, there is a lot of research being done on how to precisely control where and when PROTACs get active in the body (Nalawansha & Crews, 2020). Although PROTAC technology has advanced significantly, it has become evident that not all proteins are equally susceptible to PROTAC-mediated degradation. Proteins localised to certain subcellular compartments, including the mitochondria, Golgi apparatus, lysosome as well as multipass transmembrane proteins and extracellular proteins, often exhibit resistance to PROTAC-mediated degradation (Burslem & Crews, 2020),(Gao et al., 2020). Around the turn of the decade, a new angle of TPD was discovered, it involved the innate lysosomal degradation pathways. Through either extracellular receptors with the help of lysosome targeting chimeras (LYTACs) (Banik et al., 2020) or through hijacking the innate macro-autophagy (from now on referred to as autophagy) mechanism with autophagy-targeting chimeras (AUTOTACs and AUTACs) (Ji et al., 2022; Takahashi et al., 2019) or autophagosome-tethering compounds (ATTECs) (Li, Wang, et al., 2019; Li, Zhu, et al., 2019). AUTOTACs, AUTACs and ATTECs make use of the autophagy system, an intracellular recycling system first discovered in yeast (Takeshige et al., 1992). It allows for

balanced energy and resource distribution during nutrient stress, and it also plays a housekeeping role in removing misfolded or aggregated proteins, clearing damaged organelles, as well as eliminating intracellular pathogens (Glick et al., 2010). It achieves this by generating an intracellular membrane structure called a phagophore, which can engulf its target, thereby forming a so-called autophagosome. This autophagosome then sends its contents for lysosomal degradation (Glick et al., 2010). During autophagosome formation, proteins from the ATG8 family (e.g., LC3(B-II) and GABARAP) get incorporated into the membrane on both the internal and external sides of the membrane. These two proteins serve as anchors to which the to-be-degraded proteins, lipids, organelles, pathogens, or aggregates get attached (Glick et al., 2010). This attachment happens through LC3-interacting regions (LIR). Under normal conditions, proteins like sequestosome 1 (p62) (or NBR1, TOLLIP, and OPTN) facilitate this binding through their LIR (Klionsky et al., 2021; Zellner et al., 2021). These proteins are known as selective autophagy receptors (SARs). Additionally, these SARs have other binding domains, which include ubiquitin binding domains or direct cargo motifs (Zellner et al., 2021). This helps the selective autophagy receptor connect the cargo to the growing phagophore (Figure 1). Autophagy has proven to be essential for the homeostasis of the cell. Autophagy dysfunction is then also often linked to many pathologies, including cancer as well as cardiovascular, metabolic, renal, pulmonary, neurodegenerative, musculoskeletal and ocular disorders (Klionsky et al., 2021; Sarkar et al., 2021). While PROTACs and the ubiquitin-proteasome system are efficient in degrading soluble short-lived proteins, autophagy-mediated TPD strategies offer quite some additional advantages, most notably the ability to degrade large protein complexes, aggregates, or even entire organelles (Paudel et al., 2022). This opens up promising therapeutic avenues for conditions such as neurodegenerative diseases characterized by protein aggregates, or cancers where mitochondrial dysfunction contributes to disease progression (Lee, Sung, et al., 2023; Zong et al., 2024).

This literature review aims to qualitatively examine and compare emerging autophagy-mediated TPD technologies (i.e., LYTACS, AUTOTACs, AUTACs and ATTECs), highlighting their mechanisms of action, advantages, limitations, and therapeutic potential (see Table 1).

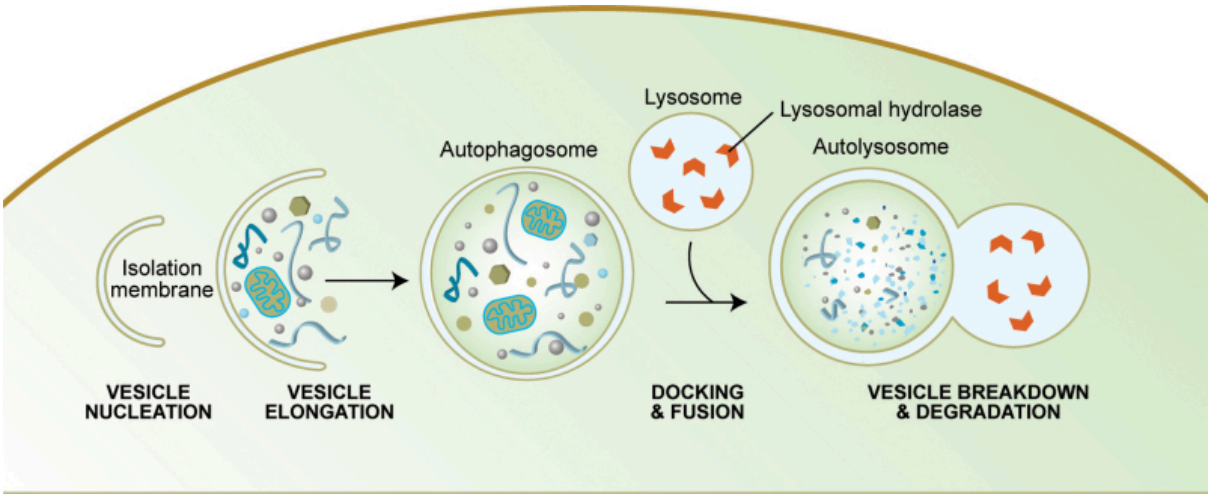

Figure 1. Schematic representation of autophagy

Meléndez, A., & Levine, B. (n.d.). Figure 1, Schematic diagram of the steps of autophagy. - WormBook - NCBI Bookshelf.

https://www.ncbi.nlm.nih.gov/books/NBK116074/figure/autophagy_figure1/

AUTOTACS & their applications.

AUTOTAC technology makes use of a chimeric design in which a target-binding ligand (TBL) directs the compound to the protein of interest (POI), while an autophagy-targeting ligand (ATL) engages the autophagy receptor p62 (sequestosome 1), through mimicking the N-degron pathway, thereby facilitating selective degradation (Ji et al., 2022). The N-degron pathway is a proteolytic system where proteins get targeted for degradation based on the identity of their N-terminal amino acid (N-degron) (Varshavsky, 2019). In the context of AUTOTACs, this pathway is mimicked by using arginine-mimicking autophagy-targeting ligands (ATLs). Upon recognition of the arginine N-degron (Arg/N-degron) ATL through its own ZZ domain, p62 undergoes a conformational change that exposes both its PB1 domain, responsible for self-polymerization, and its LC3-interacting region (Berkamp et al., 2020). This p62 to formin a complex with the target protein (aggregate) and enables p62 interaction with LC3 on the phagophores, eventually leading to lysosomal degradation (Lee, Sung, et al., 2023). (Figure 2)

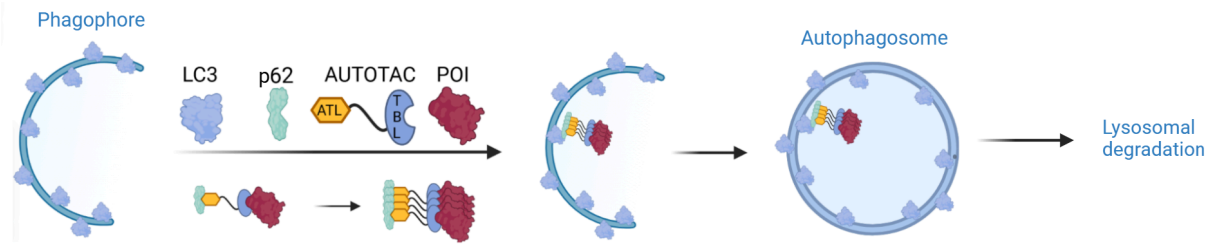

Figure 2. Schematic representation of AUTOTACs.
The AUTOTAC uses its POI-specific TBL to bind to the POI, and its ATL to bind to p62 (ATLs are often the same for different AUTOTACs), This complex can now self-polymerise and through a conformational change in p62 it can now bind to LC3 on the forming phagophore.

The AUTOTACS have demonstrated to be an effective form of TPD in, for example, Parkinson's disease (PD) (Lee, Yoon, et al., 2023; Lee, Sung, et al., 2023). In PD, α-synuclein aggregates are one of the hallmarks of the disease; they behave as neurotoxic proteins and significantly progress the disease (Mehra et al., 2019). The AUTOTAC ACT161 has been shown to selectively degrade α-synuclein aggregates in both in vitro and in vivo models (Lee, Sung, et al., 2023). ATC161 is designed with a TBL named anle138b that binds oligomeric α-synuclein (pre-)aggregates and has been shown to degrade α-synuclein aggregates at a half degradation concentration (DC50) of 100nm, leaving its monomeric form intact (Lee, Sung, et al., 2023). This selectivity is critical, as it allows the specific degradation of pathogenic α-synuclein aggregates while preserving its functional monomeric form. Furthermore, the study by Lee, Sung et al. demonstrates that ATC161 mitigates the mitotoxic effects of α-synuclein, inhibits DNA damage, and prevents the activation of downstream apoptotic signaling. By reducing the intracellular α-synuclein concentration, it indirectly also reduces the extracellular concentration of α-synuclein, thereby limiting the spread of synucleinopathy and enhancing its potential clinical relevance (Lee, Sung, et al., 2023). The study of Ji et al. (2022) looked into a similar p62-dependent AUTOTACs, they identified the critical residues in the p62 ZZ domain's N-degron recognition, being Phe168, Arg139, Ile127, Asp129, Asp147, and Asp149. Using this knowledge, they identified several p62-ZZ ligands that could serve as ATLs to be used in the AUTOTAC synthesis. In this study, the researchers linked a known TBL for one of their POIs to one of their newly

generated ATLs with repeating polyethylene glycol (PEG), to create a functional AUTOTAC. PEG was used given its favorable pharmacokinetic properties, such as high solubility (Ebrahimi & Samanta, 2023). They demonstrated the potential of AUTOTAC-based degradation for intracellular oncoproteins like estrogen receptor beta (ERβ), androgen receptor (AR), and methionine aminopeptidase 2 (MetAP2). PHTPP, vinclozolin, and fumagillol were used as TBLs, respectively, all showing DC50 values within the nanomolar range (Ji et al., 2022). But it also showed clear in vitro degradation of mutant tau, not only validating AUTOTAC's viability as a targeted degrader of intracellular oncoproteins but also showing its potential in Alzheimer's disease (AD) treatment. As more research on the different SARs (e.g. NBR1, TOLLIP, and OPTN) is done, more specific ATL's will likely be developed in the next few years (Johansen & Lamark, 2019). As of our current understanding, the AUTOTAC technology has been the most promising when it comes to clearing aggregated proteins (Lee, Sung, et al., 2023; Mieland et al., 2022). A common hallmark across many neurodegenerative diseases (Wilson et al., 2023). This makes the AUTOTAC technology a promising therapeutic strategy in treating these diseases.

AUTACS & their applications:

The AUTAC technology is very similar to that of the AUTOTAC technology, the only difference being that AUTAC technology indirectly marks the proteins/organelles for degradation through K-63 poly-ubiquitination (Vatté et al., 2024; Wang et al., 2024). A mechanism innately used to tag pathogens for lysosomal degradation (Ito et al., 2013; Arimoto, 2015). This happens after the AUTAC compound induces a POI-guanosylation modification, leading to K63 polyubiquitination of the POI by an (undefined) E3 ligase to which a selective autophagy receptor like p62 gets recruited (Dósa & Csizmadia, 2022). This facilitates the connection with the autophagosome, eventually leading to lysosomal degradation (Takahashi et al., 2019). (Figure 3)

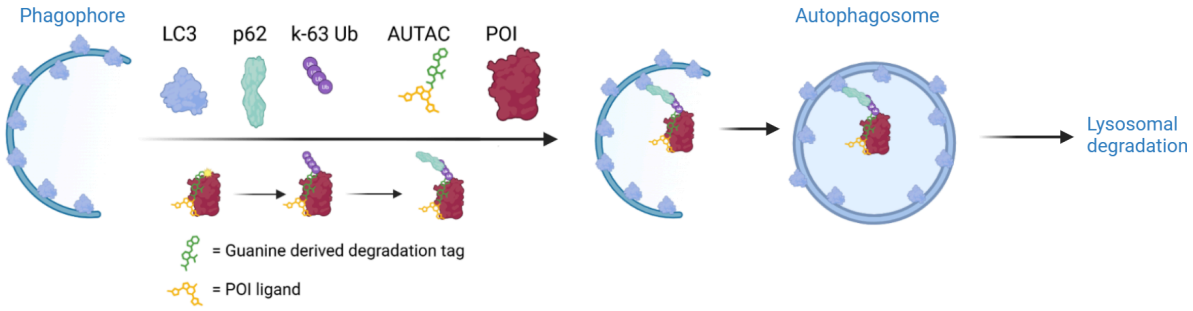

Figure 3. Schematic representation of AUTACs

The AUTAC consists of two moieties: the guanine-derived degradation tag, which induces POI-guanosylation, and the POI ligand, which allows for binding to the POI. After guanosylation, the POI gets ubiquitinated by an E3 ligase, which allows p62 to bind with its ubiquitin-binding domain, inducing a conformational change in p62, allowing it to bind to LC3 on the forming phagophore.

Mitochondria-targeting AUTAC (mito-AUTAC) compounds have been shown to induce mitophagy and improve mitochondrial functioning in DS cells and act in a parkin-independent way, which makes it viable in tissues or syndromes in which Parkin is disabled (parkinson's & several cancers) (Takahashi et al., 2019). In the study from Wang et al. (2024), they proved the tumor specific degradation of the immunosuppresive tumor-associated protein indoleamine 2,3-dioxygenase (IDO). Since the AUTAC technology still struggles with poor

specific distribution and low bioavailability due to poor solubility and high polarity. The researchers believed that through supramolecular self-assembly of so-called nano-AUTACS, they could alleviate these issues and improve tumor-specific IDO degradation. To test this, they designed the AUTAC GN, consisting of an IDO-binding ligand (NLG8189) with a purine-based degradation tag inducing K63 polyubiquitination, essentially forming an IDO degrader (Y. Wang et al., 2024). They co-assembled this with methotrexate (MTX), a well-established chemotherapy agent (Widemann & Adamson, 2006) to further improve the effectiveness of the treatment. Through transmission electron microscopy, dynamic light scattering, and ultraviolet analysis, a stable intermolecular interaction between GN and MTX was observed, forming the GM nanoparticles (GM NP's). Upon entering the tumor microenvironment (TME) , the two components disassembled due to the increased acidity (Boedtkjer & Pedersen, 2019). This allows the two components to become active, thereby improving the specificity of active compound distribution (being only in the TME) and thereby also reducing off-target effects. This so called combination therapy shows great potential both in vitro aswel as in vivo (Y. Wang et al., 2024). AUTACs and especially nano-AUTACs have demonstrated that through clever design, a specific and directed form of TPD is possible, making the AUTAC technology a promising field that deserves more attention and sustained research investments.

ATTECS & their applications:

ATTECs represent the most direct approach to autophagy-mediated TPD. These small molecules are composed of a ligand that binds to LC3B and another part that targets the POI. Together, they facilitate the selective degradation of the POI (Figure 4).

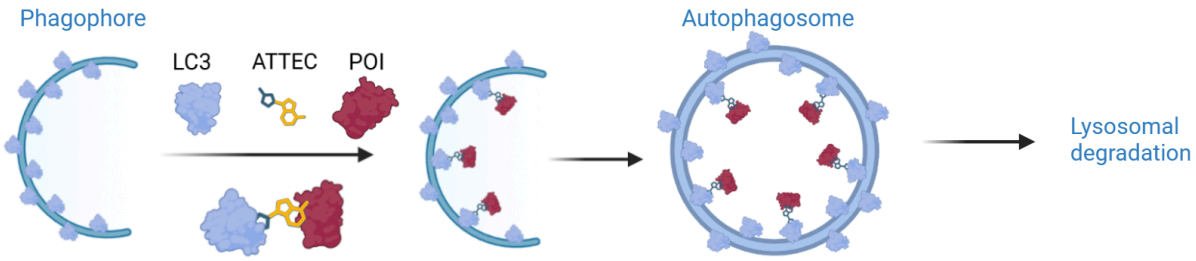

Figure 4. Schematic representation of ATTECs.
The ATTEC consists of two moieties, one binding to the POI (yellow part) and one binding to LC3(B) (blue part), allowing for effective tethering of the cargo to the forming phagophore.

Mitochondrial dysfunction or impaired mitophagy plays a role in the pathogenesis of various diseases (Zong et al., 2024). Even though small-molecule compounds that induce mitophagy have already been developed, they often act through damaging the mitochondria rather than improving and enhancing underlying mitophagic mechanisms (Frank et al., 2012; Georgakopoulos et al., 2017; Kim et al., 2007). In the study by Tan et al. (2023), the potential of ATTECs to mark mitochondria for degradation was explored. First proof of concept was established using the FRB-FKBP heterodimer system (a well-established chemically induced oligomerisation system) to tether LC3B to a mitochondrial protein (TSPO), resulting in enhanced mitophagy (after adding the dimerization compound rapamycin) (Inobe & Nukina, 2016). This success eventually led them to designing two chimeric compounds, of which mT1, consisting of 2-phenylindole derivative (a known TSPO ligand) and a previously

reported LC3 interaction compound (GW5074) (Li, Wang, et al., 2019) via a 10-carbon chain. Cells treated with this mT1 compound showed increased mitophagy through coupling (damaged) mitochondria to the forming phagophore (Tan et al., 2023). These mitophagy-inducing ATTEC compounds are called mito-ATTECS. These results were experimentally determined using mito-Keima, a fluorescent protein that gets directed to the mitochondrial matrix, where it emits green fluorescence (440nm) under normal conditions while shifting to a red fluorescence (586nm) when entering the acidic environment of the lysosome. This change in fluorescence allows mitophagy activation to become quantitatively detectable. Not only does mT1 induce mitophagy by itself when added to cells, but it even shows increased mitophagy after CCCP-induced mitochondrial damage. This indicates an improved degradation of damaged mitochondria compared to normal functioning mitochondria. Given the role of impaired mitophagy in neurodegenerative disease pathogenesis, it is promising that mT1 inhibited apoptosis in MPP+ treated SH-SY5Y cells, a cellular model for Parkinson's disease. It also attenuated mitochondrial defect-related phenotypes in a DS organoid model. The study of Liao et al. (2023) also provides proof of concept of degrading α-synuclein through aptamer ATTECs. These aptamers consist of single-stranded RNA or DNA that can specifically bind a POI (Liao et al., 2023). This finding broadens the scope in which ATTECs can be used. Another study from Z. Liu et al. (2023), also looked into these so-called mito-ATTEC compounds. Whilst using a similar experimental setup, they discovered the cytotoxic effect of these compounds on tumor cells (Liu et al., 2023). Especially melanoma cell lines (especially A375 (IC50 = 11.9μM)) were found to be sensitive to the mito-ATTEC compound. This effect most likely occurs due to elevated LC3 levels in these melanoma lineages as well as their upregulated autophagy mechanisms making them more vulnerable to this lethal mitophagy (Z. Liu et al., 2023; Pangilinan et al., 2024). ATTEC compounds have not only been shown to be useful in cancer-, PD- and DS-treatment, but a study from Li, Wang, et al., (2019) and another study from Tabrizi et al. (2019) proved that it is also capable of tethering and degrading mutant huntingtin (mHTT). The ATTEC in question was derived from a microarray screening for pathogenic mHTT binding and LC3B binding. Two lead compounds (10O5 & AN2) that could pass the blood-brain barrier were found. They identified the molecule interacted with the mHTT's expanded polyQ stretch leaving WT HTT unaffected (Li, Wang, et al., 2019). Given this information the compound was also tested on another polyQ expansion protein that causes spinocerebellar ataxia 3 (SCA3) where it also effectively depleted the mutant protein leaving the WT intact (Li, Wang, et al., 2019). All previous studies made use of the direct LC3 binding domains but Schwalm et al. (2024) were not able to reproduce some of these ATTEC compounds' proclaimed LC3 binding.They did further clarify that not only LC3 but also GABARAP(L1 & L2), another ATG8 family protein, can be used to design ATTECs (Schwalm et al., 2024). Furthermore, a study done by Fu et al. (2021) demonstrates the ability to effectively degrade lipid droplets (LD) with the help of ATTECs. To do this, they linked an LC3 binding compound to an LD detection probe (called SIII or SIV). There are, however, some safety concerns with the current LD detection probes used, high concentrations have proven to be carcinogenic (*Carcinogenesis Bioassay of C.I. Solvent Yellow 14 (CAS No. 842-07-9) in F344/N Rats and B6C3F1 Mice (FEED Study)*, 1982). So further optimisation of these probes is still necessary. ATTECs have been shown to be a very direct form of autophagy-mediated TPD. This, combined with their ability to target LD (Fu et al., 2021) and their sustained degradation at low concentrations (Li et al., 2019; Tan et al., 2023) makes them a viable strategy to continue developing.

LYTACS & their applications:

LYTACS are not officially autophagy-mediated degraders, but they do behave in a very similar way by sending their designated cargo to the lysosome for degradation. One major distinction and immediate advantage between LYTACS and other autophagy-mediated TPD strategies is that LYTACS can facilitate the degradation of both membrane-bound and extracellular proteins (Banik et al., 2020). They act through coupling the POI to a lysosome-shuttling receptor such as the cation-independent mannose-6-phosphate receptor. Upon activation of this receptor, the LYTAC and POI get encapsulated through endocytosis and sent to the lysosome (Figure 5).

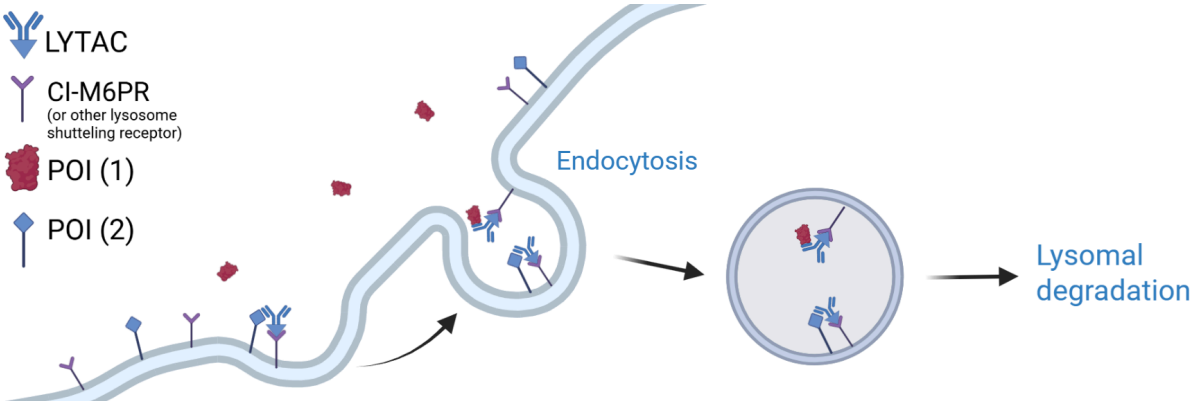

Figure 5. Schematic representation of LYTACs.
LYTACs consists of a lysosome-shuttling receptor ligand (such as manose-6-phosphate for the CI-M6PR) and a binding domain for the POI. When bound to the lysosome-shuttling receptor, the complex undergoes endocytosis, after which it is taken to the lysosome for degradation.

The study of Banik et al. (2020) looked into designing LYTACs through the cation-independent mannose-6-phosphate receptor (CI-M6PR or IGF2R). They concluded that a single regular 6-phosphoester ligand, like in mannose-6-phosphate (M6P), could undergo hydrolysis in human serum and get cleared by macrophages through endocytosis, while a N-carboxyanhydride (NCA)-derived glycopolypeptide with many serine-O-mannose-6-phosphonate (M6Pn) groups is better at activating the CI-M6PR receptor (Banik et al., 2020). This meant the researchers now had a multivalent presentation on a biocompatible, phosphatase-inert and modular scaffold. During the development of these LYTACs, it was also discovered that surface-receptor recycling of CI-M6PR is the rate-limiting step. And that a combined gene knockout of EXOC1 & EXOC2 (proteins involved in exocytosis) resulted in a strong decrease (60-75%) of cell surface CI-M6PR receptor, indicating the importance of the exocyst complex in LYTAC-mediated extracellular degradation (Banik et al., 2020). They found that when you combine an IgG antibody with an M6pn-bearing glycopolypeptide, you could degrade the antibody-targeted extracellular protein between 10-100 times faster, making use of the CI-M6PR lysosome targeting (Banik et al., 2020). In this same study, they were able to degrade apolipoprotein E4 (ApoE4) (a protein involved in AD pathogenesis) 13 times faster than their control (without LYTACs). Additionally, they managed to degrade a membrane-bound driver of cancer proliferation, epidermal growth factor receptor (EGFR), by up to 70% without significantly altering CI-M6PR levels. This was achieved with antibody-based LYTACs. This degradation was observed after 3h, peaked around 12-24h, and lasted for at least 72h. The study above utilised glycoproteins, which can be hard to prepare and produce on a large scale (Zhang et

al., 2022; Stevens et al., 2023). Mikitiuk et al. (2023) tried to design a pure protein LYTAC as an alternative for the glycopolypeptide strategy. They designed antibodies based on IGF2 for the transmembrane protein PD-L1 a protein that suppresses T-cells and is involved in tumor proliferation (Dong et al., 2018). Here they discovered a 30% growth inhibition of tumor cells depending on the antibodies' design. Other types of LYTACS have also been in development, for example, RNA or DNA-based aptamers have been used to make a low molecular weight LYTAC, these have been shown to efficiently target and degrade extracellular (PDGF) and membrane (PTK7) proteins (Wu et al., 2023). Another example of aptamer-based LYTACs lies in degrading vascular endothelial growth factor (VEGF), a driver of neovascular ocular diseases (NODs). NODs are the leading cause of irreversible vision impairment worldwide (Z. Wang et al., 2018) and in the study of Zhou et al. (2024) they developed achimeric LYTAC compound V5 and showed that it time-dependently degrades VEGF in vitro (Zhou et al., 2024). Besides using the CI-M6PR, other receptors have also been discovered for LYTAC generation. Another widely studied receptor is the Liver-specific asialoglycoprotein receptor (ASGPR) for which N-acetylgalactosamine (tri-GalNAc) is used for receptor binding. Other utilised receptors include: integrin (Zheng et al., 2022), the angiopep-2 receptor (Howell et al., 2024), LRP-1, CXCR7 (Pance et al., 2022) ,TfR1 (D. Zhang et al., 2024), ZNRF3 (Marei et al., 2022), RNF43 (Cotton et al., 2021) and surface scavenger receptors (Zhu et al., 2023). LYTACs have proven to degrade the previously undegradable extracellular proteins or membrane-bound proteins efficiently. This makes the LYTAC technology a viable new TPD strategy to be added to the toolbox.

Conclusion and discussion:

While PROTACS excel at the rapid turnover of soluble (onco)proteins, autophagy-based degraders extend the degradable substrate space to include UPS-resistant aggregates, damaged organelles, and via LYTAC membrane-bound and extracellular proteins that are inaccessible to the proteasomal system. AUTOTACs have been shown to directly degrade pathogenic protein aggregates such as α-synuclein (Lee, Sung, et al., 2023) or degrade aggregation-prone mutant tauP301L (Ji et al., 2022) by mimicking the Arg/N-degron pathway and activating p62. The ATTEC compounds have shown to clear mutant Huntingtin protein (Li et al., 2019) and to clear lipid droplets and induce mitophagy in disease models (Tan et al., 2023). And the LYTAC technology manages to degrade disease-relevant extracellular proteins (e.g. ApoE4, PD-L1) by engaging with several lysosome-targeting receptors (e.g. CI-M6PR or ASGPR).

The techniques covered in this review were developed in parallel, even though they have a lot in common, they each have their strengths and weaknesses (see Table 1). Given their different mode of action, they interact with different proteins and therefore can hold relevance in different diseases, making their co-existence justified.

Despite their therapeutic potential, autophagy-targeting chimeras face a number of unresolved technical and biological challenges. One of them is that nearly all presented studies are still in the proof-of-concept stage, experimenting with animal and in vitro models and are still a long way from clinical research (Zhong et al., 2024). So only time will tell how well these studies translate to (pre-)clinical trials.

Another point of discussion is the current imperfections when it comes to the drug-like properties of many autophagy-mediated TPD. It has already shortly been discussed that the glycoproteins used in LYTACs are ‘difficult to prepare and produce on a large scale’ (Mikitiuk et al., 2023). This slows down the development and discoveries made in this field. Luckily, other alternatives are being developed (think of aptamers (Wu et al., 2023) or all-protein antibodies (Dong et al., 2018) but they have their disadvantages too and are still in a very early stage of development (News-Medical, 2019). One of the issues with using antibodies is that they are not orally administrable, having the LYTACs to be administered intravenously. This is a recurring issue also with AUTACs, given they can be relatively large and thus have limited (oral) bioavailability or BBB penetration (Zhong et al., 2024). The ATLs in AUTOTACs are still relatively lipophilic, which causes there to be an increased risk for off-target effects (Ji et al., 2022). However, on a positive note, the ATC161 AUTOTAC has shown promising results after pharmacokinetic analysis, showing high solubility and 10% brain penetration (Lee, Sung, et al., 2023) making it a promising compound to continue researching. Concerning the lipophilicity problem of the ATLs, it has to be considered that there are many more SARs for wich ATL can be designed (Johansen & Lamark, 2019), potentially resolving these issues in the future.

As Schwalm et al. (2024) stated, many of the ATTEC compounds currently being developed showed weak binding to LC3, indicating there is still a lot to be improved. This same study also emphasized that due to the field being relatively new, no standard (control) experiments have been established, which can make results hard to interpret or reproduce (Schwalm et al., 2024). Another effect of this field of research being relatively new is that there is still little known about the off-target effects of many of these compounds. All studies include basic cytotoxicity tests, and some even go as far as screening for connected genes through CRISPR interference (Banik et al., 2020), but overall, off-targets are just not well enough understood (Ji et al., 2022). To solve the LC3 binding problem mentioned above, high-throughput fragment-based drug discovery and (virtual) hit optimization should be adapted and standardized in generating new compounds, as mentioned in the paper from Schwalm et al. (2024). To further evaluate off-target effects in the TPD field Scholes et al. (2021) suggest looking into expression proteomics and to address the genes necessary for target degradation, CRISPR interference screenings can be used. Additionally, a version of an autophagy-mediated TPD compound (e.g. ATTEC) with a tagging probe can be used together with activity-based protein profiling techniques to determine off-target binding sites (Kuljanin et al., 2021; Niphakis & Cravatt, 2024).

Another point of discussion is the recyclability of many of these compounds. It is pretty clear LYTACs and AUTACs get degraded in the process (Banik et al., 2020; Mikitiuk et al., 2023; Takahashi et al., 2019). As a result, continuous dosing may be necessary to effectively combat the disease, potentially leading to high treatment costs. When it comes to AUTOTACs and ATTECs, it is not known yet if the compounds act more catalytically or also get consumed in the process. More mechanistic studies should be done; however, ATTEC compounds do show sustained degradation with relatively low concentrations (Li et al., 2019) (Tan et al., 2023). This lack of recyclability is a major disadvantage of autophagy-mediated TPD, especially when it's compared with the well-established PROTAC technology, since these act as catalysts (Bondeson et al., 2015).

It is also important to consider that many diseases in which autophagy-mediated TPD holds clinical promise are themselves characterized by dysregulated autophagy, which could impact the effectiveness of these therapies. This dysregulated autophagy has been observed in PD (Menzies et al., 2017; Sarkar et al., 2021), AD (Menzies et al., 2017), Huntington's disease (Menzies et al., 2017), and also Down syndrome (Tan et al., 2023). This might lead to the ineffectiveness of certain compounds for people who have an essential protein dysregulated. Prior to developing a novel autophagy-mediated TPD compound, transcriptomic screening should be performed to determine whether certain strategies may be less applicable in that specific disease. Another issue with degradation through the lysosome in neurodegenerative diseases is that, when the POI is located in neuronal axons or dendrites, it must undergo a long retrograde transport process back to the soma before it can be degraded, reducing efficiency.

Currently, the rate-limiting step in LYTAC technologies often lies in the recycling of the receptor (Banik et al., 2020) and momentarily this puts up a barrier holding off further conversion rate improvements. Another issue with the CI-M6PR is that the innate M6P-signaling can occupy the receptor (Ahn et al., 2023). To improve lysosomal degradation of target proteins, the next generation of LYTACs should look into orthogonal binding sites on CI-M6PR to outcompete other M6P-tagged molecules (Ahn et al., 2023).

In the future, autophagy-mediated TPD will continue to be developed. Even though the techniques are promising, there is still a lot to be improved. Further chemical optimization of degradation tags and linkers can for example, improve solubility and cell entry. A good example of this optimisation is stated in the paper of Takahashi et al. (2023), where second-generation AUTACS were made using L-cys as a connector. They optimized the length of the linker and guanine degradation tag. Another way of improving AUTACs in the future is to look into nanocarriers to deliver the drug to predetermined locations in the body. A nice example of this is the ‘nano-AUTAC’ paper from Wang et al. (2024) where they used a chemotherapy agent, methotrexate, which was attached through non-covalent bonds. This molecule guided the AUTAC to the tumors and when it entered the more acidic TME, the two separated and fulfilled their ‘tasks’. If autophagy-mediated TPD is going to become clinically relevant a more targeted delivery will be essential in minimising off-target effects.

In general, the delivery of autophagy-mediated TPD into either a pre-determined location in the body as well as the entry into the intracellular environment is pretty poorly described. With recent advances in nanocarrier technologies (N. Wang et al., 2018) for example in the passing of the blood brain barrier through receptor-mediated transcytosis (Haqqani et al., 2024) a highly potent medicine for NDs could be developed. Especially when taking the recyclability limitations of both LYTACs and AUTACs into consideration, a more directed delivery could prove essential in the development of future medicine in this class. Another possible approach could be to find a way to improve spatiotemporal control of the drugs through, for example, light-induced activity, a technique currently being developed in PROTACs (Nalawansha & Crews, 2020) (Jin et al., 2020).

In conclusion, autophagy-mediated TPD represents a rapidly advancing and promising frontier in therapeutic development. By making use of the cell’s innate lysosomal degradation machinery, these techniques offer a robust and versatile platform for the selective removal of intracellular, membrane-bound, or even extracellular disease-driving

proteins. The clinical potential is especially promising in the context of ND's cancer and genetic disorder, where dysfunctional protein homeostasis is a key pathogenic factor. Think of the AUTOTAC ACT161 which is able to degrade α-synuclein aggregates, mito-ATTACs and mito-AUTACs which have shown to improve the mitochondrial quality control important in PD cancer and even Down syndrome. Meanwhile, LYTACs unlocked the degradation of previously hard to control extracellular and membrane-bound proteins. Despite ongoing challenges regarding pharmacokinetics, off-target effects, and targeted delivery, the future for these emerging therapies remains highly promising.

Table 1: TPD technologies overview.

	PROTACs	AUTOTACs	AUTACs	ATTECs	LYTACs
Targeting Mechanism	Binds POI and recruits an E3 ligase	Binds POI and p62 (autophagy cargo receptor)	Binds POI and tags it with a degradation signal	Binds POI and ATG8, tethering POI to the phagophore	Binds extracellular or membrane-bound POI and lysosome-shuttling receptor.
Degradation Pathway	Ubiquitin-Proteasome System (UPS)	Autophagy pathway	Autophagy pathway	Autophagy pathway	Endocytosis → lysosome
Tagging Strategy	E3 ligase recruitment leads to ubiquitination	-	Small molecule confers K63 ubiquitination-like tag	-	-
Target Protein Type	Primarily intracellular, soluble proteins	Intracellular proteins, including aggregates	Intracellular proteins or organelles	Intracellular soluble proteins or organelles	Extracellular or membrane-bound proteins
Molecular Design	Heterobifunctional molecule: POI ligand + E3 ligase ligand + linker	Heterobifunctional: POI ligand (+ linker) + p62 ligand	Heterobifunctional molecule: POI ligand (+ linker) + guanine-derived degradation tag	Heterobiifunctional: POI ligand (+ linker) LC3 ligand	Heterobifunctional Antibody(or other POI ligand) + lysosome-shuttling receptor ligand (such as M6P)
Receptor/Recruiter Used	E3 ligases (e.g., VHL, CRBN, MDM2)	p62/SQSTM1 (via ZZ domain)	P62 or other receptors with ubiquitin-binding domain (mimicking K63 ubiquitin signal)	LC3 (key autophagosome marker)	CI-M6PR or ASGPR for lysosomal trafficking
Catalytic Turnover	Yes	Potentially yes	No	Potentially yes	No (usually stoichiometric uptake)
Cellular Permeability	Small molecule, generally	(Small) molecule, cell-permeab	Small molecule, cell-permeabl	Small molecule, cell-permeable	Typically non-permeable (biologics) however,

	cell-permeable	le	e		permeability is not necessary.
Clinical Status	Several in clinical trials (e.g., ARV-110)	Preclinical	Preclinical	Preclinical	Preclinical
Disease with potential therapy.	Cancer, AD, Huntington, Inflammation and SARS-CoV-2	Cancer, PD, AD	Cancer, PD, DS	Cancer, Huntington, Fatty liver disease, and Obesity	Cancer, AD, NODs
Advantages	Well-established, potent, and tunable	Shows great potential in degrading aggregated proteins	Has been shown to work in complex with chemotherapy agents. (nano-AUTACs)	Small molecule, decent cell permeability and good plasma half-life.	Enables TPD of secreted or membrane proteins
Limitations	UPS limitations (e.g., size of complexes)	Lipophilic off-targets cause risk of off-target effects.	Current designs have poor solubility and bioavailability (nano-AUTAC could provide the outcome)	Often weak binding to LC3 so not highly efficient.	Receptor turnover rate can be limiting. Often based on antibodies requiring intravenous administration.
Examples	ARV-110 (androgen receptor degrader)	ATC161 targeting α-synuclein aggregates	methotrexate based nano-AUTAC	ATTEC targeting mutant huntingtin (mHTT)	LYTACs targeting PD-L1, EGFR

Table 1. Comparative overview of all different TPD strategies.
(references for potential PROTAC therapies: (Liu et al., 2022; Fiorillo et al., 2021; Chu et al., 2016)

AI declaration:

During the writing of this thesis, the generative AI ChatGPT was used to help with improving overall flow, correcting spelling and grammar mistakes, and assisting to look for sources. Everything provided by the AI was always rigorously checked before it was used in the writing of this thesis.

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References:

Ahn, G., Riley, N. M., Kamber, R. A., Wisnovsky, S., Von Hase, S. M., Bassik, M. C., Banik, S. M., & Bertozzi, C. R. (2023). Elucidating the cellular determinants of targeted membrane protein degradation by lysosome-targeting chimeras. *Science*, *382*(6668). https://doi.org/10.1126/science.adf6249

Akwa, Y., Di Malta, C., Zallo, F., Gondard, E., Lunati, A., Diaz-De-Grenu, L. Z., Zampelli, A., Boiret, A., Santamaria, S., Martinez-Preciado, M., Cortese, K., Kordower, J. H., Matute, C., Lozano, A. M., Capetillo-Zarate, E., Vaccari, T., Settembre, C., Baulieu, E. E., & Tampellini, D. (2022). Stimulation of synaptic activity promotes TFEB-mediated clearance of pathological MAPT/Tau in cellular and mouse models of tauopathies. *Autophagy*, *19*(2), 660–677. https://doi.org/10.1080/15548627.2022.2095791

Arimoto, H. (2015). Roles of 8-nitro-cGMP in autophagy regulation. *BMC Pharmacology and Toxicology*, *16*(S1). https://doi.org/10.1186/2050-6511-16-s1-a14

Banik, S. M., Pedram, K., Wisnovsky, S., Ahn, G., Riley, N. M., & Bertozzi, C. R. (2020). Lysosome-targeting chimaeras for degradation of extracellular proteins. *Nature*, *584*(7820), 291–297. https://doi.org/10.1038/s41586-020-2545-9

Berkamp, S., Mostafavi, S., & Sachse, C. (2020). Structure and function of p62/SQSTM1 in the emerging framework of phase separation. *FEBS Journal*, *288*(24), 6927–6941. https://doi.org/10.1111/febs.15672

Boedtkjer, E., & Pedersen, S. F. (2019). The acidic tumor microenvironment as a driver of cancer. *Annual Review of Physiology*, *82*(1), 103–126. https://doi.org/10.1146/annurev-physiol-021119-034627

Bondeson, D. P., Mares, A., Smith, I. E. D., Ko, E., Campos, S., Miah, A. H., Mulholland, K. E., Routly, N., Buckley, D. L., Gustafson, J. L., Zinn, N., Grandi, P., Shimamura, S., Bergamini, G., Faelth-Savitski, M., Bantscheff, M., Cox, C., Gordon, D. A., Willard, R. R., . . . Crews, C. M. (2015). Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nature Chemical Biology*, *11*(8), 611–617. https://doi.org/10.1038/nchembio.1858

Burslem, G. M., & Crews, C. M. (2020). Proteolysis-Targeting Chimeras as Therapeutics and Tools for Biological Discovery. *Cell*, *181*(1), 102–114. https://doi.org/10.1016/j.cell.2019.11.031

Carcinogenesis Bioassay of C.I. solvent Yellow 14 (CAS No. 842-07-9) in F344/N rats and B6C3F1 mice (FEED Study). (1982, September 1). PubMed. https://pubmed.ncbi.nlm.nih.gov/12778210/

Chen, Q., Munoz, E., & Ashong, D. (2024). Insight into Recent Advances in Degrading Androgen Receptor for Castration-Resistant Prostate Cancer. *Cancers*, *16*(3), 663. https://doi.org/10.3390/cancers16030663

Chu, T., Gao, N., Li, Q., Chen, P., Yang, X., Chen, Y., Zhao, Y., & Li, Y. (2016). Specific knockdown of endogenous TAU protein by Peptide-Directed Ubiquitin-Proteasome degradation. *Cell Chemical Biology*, *23*(4), 453–461. https://doi.org/10.1016/j.chembiol.2016.02.016

Cotton, A. D., Nguyen, D. P., Gramespacher, J. A., Seiple, I. B., & Wells, J. A. (2021). Development of Antibody-Based PROTACs for the degradation of the Cell-Surface immune checkpoint protein PD-L1. *Journal of the American Chemical Society*, *143*(2), 593–598. https://doi.org/10.1021/jacs.0c10008

Dong, P., Xiong, Y., Yue, J., Hanley, S. J. B., & Watari, H. (2018). Tumor-Intrinsic PD-L1 signaling in Cancer Initiation, Development and Treatment: Beyond immune evasion. *Frontiers in Oncology*, *8*. https://doi.org/10.3389/fonc.2018.00386

Dósa, A., & Csizmadia, T. (2022). The role of K63-linked polyubiquitin in several types of autophagy. *Biologia Futura*, *73*(2), 137–148. https://doi.org/10.1007/s42977-022-00117-4

Ebrahimi, S. B., & Samanta, D. (2023). Engineering protein-based therapeutics through structural and chemical design. *Nature Communications*, *14*(1). https://doi.org/10.1038/s41467-023-38039-x

Fiorillo, A., Morea, V., Colotti, G., & Ilari, A. (2021). Huntingtin ubiquitination mechanisms and novel possible therapies to decrease the toxic effects of mutated huntingtin. *Journal of Personalized Medicine*, *11*(12), 1309. https://doi.org/10.3390/jpm11121309

Frank, M., Duvezin-Caubet, S., Koob, S., Occhipinti, A., Jagasia, R., Petcherski, A., Ruonala, M. O., Priault, M., Salin, B., & Reichert, A. S. (2012). Mitophagy is triggered by mild oxidative stress in a mitochondrial fission dependent manner. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research*, *1823*(12), 2297–2310. https://doi.org/10.1016/j.bbamcr.2012.08.007

Fu, Y., Chen, N., Wang, Z., Luo, S., Ding, Y., & Lu, B. (2021). Degradation of lipid droplets by chimeric autophagy-tethering compounds. *Cell Research*, *31*(9), 965–979. https://doi.org/10.1038/s41422-021-00532-7

Gao, H., Sun, X., & Rao, Y. (2020). PROTAC Technology: opportunities and challenges. *ACS Medicinal Chemistry Letters*, *11*(3), 237–240. https://doi.org/10.1021/acsmedchemlett.9b00597

Georgakopoulos, N. D., Wells, G., & Campanella, M. (2017). The pharmacological regulation of cellular mitophagy. *Nature Chemical Biology*, *13*(2), 136–146. https://doi.org/10.1038/nchembio.2287

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *The Journal of Pathology*, *221*(1), 3–12. https://doi.org/10.1002/path.2697

Haqqani, A. S., Bélanger, K., & Stanimirovic, D. B. (2024). Receptor-mediated transcytosis for brain delivery of therapeutics: receptor classes and criteria. *Frontiers in Drug Delivery*, *4*. https://doi.org/10.3389/fddev.2024.1360302

Howell, R. A., Wang, S., Khambete, M., McDonald, D. M., & Spiegel, D. A. (2024). Bifunctional molecules that induce both targeted degradation and transcytosis of extracellular proteins in brain cells. *Journal of the American Chemical Society*, *146*(24), 16404–16411. https://doi.org/10.1021/jacs.3c13320

Hurvitz, S. A., MD, Schott, A. F., MD, X, C., MA MD PhD, Hamilton, E. P., MD, Nanda, R., MD, Zahrah, G., Hunter, N., MD, Tan, A. R., Telli, M. L., MD, Mesias, J. A., Jeselsohn, R., Munster, P. N., MD, Lu, H., Gedrich, R., Mather, C., Parameswaran, J., & Han, H. S. (2023, April 27). 24 ARV-471, a PROTAC estrogen receptor (ER) degrader in advanced ER+/Human Epidermal Growth Factor Receptor 2 (HER2)– Breast Cancer: Phase 2 expansion (VERITAC) of a Phase 1/2 study. *Cancer Network*. https://www.cancernetwork.com/view/24-arv-471-a-protac-estrogen-receptor-er-degrader-in-advanced-er-human-epidermal-growth-factor-receptor-2-her2-breast-cancer-phase-2-expansion-veritac-of-a-phase-1-2-study?

Inobe, T., & Nukina, N. (2016). Rapamycin-induced oligomer formation system of FRB–FKBP fusion proteins. *Journal of Bioscience and Bioengineering*, *122*(1), 40–46. https://doi.org/10.1016/j.jbiosc.2015.12.004

Ito, C., Saito, Y., Nozawa, T., Fujii, S., Sawa, T., Inoue, H., Matsunaga, T., Khan, S., Akashi, S., Hashimoto, R., Aikawa, C., Takahashi, E., Sagara, H., Komatsu, M., Tanaka, K., Akaike, T., Nakagawa, I., & Arimoto, H. (2013). Endogenous Nitrated Nucleotide Is a Key Mediator of Autophagy and Innate Defense against Bacteria. *Molecular Cell*, *52*(6), 794–804. https://doi.org/10.1016/j.molcel.2013.10.024

Ji, C. H., Kim, H. Y., Lee, M. J., Heo, A. J., Park, D. Y., Lim, S., Shin, S., Ganipisetti, S., Yang, W. S., Jung, C. A., Kim, K. Y., Jeong, E. H., Park, S. H., Kim, S. B., Lee, S. J., Na, J. E., Kang, J. I., Chi, H. M., Kim, H. T., . . . Kwon, Y. T. (2022). The AUTOTAC chemical biology platform for targeted protein degradation via the autophagy-lysosome system. *Nature Communications*, *13*(1). https://doi.org/10.1038/s41467-022-28520-4

Jin, Y., Lu, M., Wang, Y., Shan, W., Wang, X., You, Q., & Jiang, Z. (2020). AzO-PROTAC: Novel Light-Controlled Small-Molecule tool for protein knockdown. *Journal of Medicinal Chemistry*, *63*(9), 4644–4654. https://doi.org/10.1021/acs.jmedchem.9b02058

Johansen, T., & Lamark, T. (2019). Selective autophagy: ATG8 family proteins, LIR motifs and cargo receptors. *Journal of Molecular Biology*, *432*(1), 80–103. https://doi.org/10.1016/j.jmb.2019.07.016

Kim, E. H., Sohn, S., Kwon, H. J., Kim, S. U., Kim, M., Lee, S., & Choi, K. S. (2007). Sodium selenite induces Superoxide-Mediated mitochondrial damage and subsequent autophagic cell death in malignant glioma cells. *Cancer Research*, *67*(13), 6314–6324. https://doi.org/10.1158/0008-5472.can-06-4217

Klionsky, D. J., Petroni, G., Amaravadi, R. K., Baehrecke, E. H., Ballabio, A., Boya, P., Pedro, J. M. B., Cadwell, K., Cecconi, F., Choi, A. M. K., Choi, M. E., Chu, C. T., Codogno, P., Colombo, M. I., Cuervo, A. M., Deretic, V., Dikic, I., Elazar, Z., Eskelinen, E., . . . Pietrocola, F. (2021). Autophagy in major human diseases. *The EMBO Journal*, *40*(19). https://doi.org/10.15252/embj.2021108863

Lee, J., Sung, K. W., Bae, E., Yoon, D., Kim, D., Lee, J. S., Park, D., Park, D. Y., Mun, S. R., Kwon, S. C., Kim, H. Y., Min, J., Lee, S., Suh, Y. H., & Kwon, Y. T. (2023). Targeted degradation of α-synuclein aggregates in Parkinson's disease using the AUTOTAC technology. *Molecular Neurodegeneration*, *18*(1). https://doi.org/10.1186/s13024-023-00630-7

Lee, J., Yoon, D., Sung, K. W., Bae, E., Park, D., Suh, Y. H., & Kwon, Y. T. (2023). Targeted degradation of SNCA/α-synuclein aggregates in neurodegeneration using the AUTOTAC chemical platform. *Autophagy*, *20*(2), 463–465. https://doi.org/10.1080/15548627.2023.2274711

Li, Z., Wang, C., Wang, Z., Zhu, C., Li, J., Sha, T., Ma, L., Gao, C., Yang, Y., Sun, Y., Wang, J., Sun, X., Lu, C., Difiglia, M., Mei, Y., Ding, C., Luo, S., Dang, Y., Ding, Y., . . . Lu, B. (2019). Allele-selective lowering of mutant HTT protein by HTT–LC3 linker compounds. *Nature*, *575*(7781), 203–209. https://doi.org/10.1038/s41586-019-1722-1

Li, Z., Zhu, C., Ding, Y., Fei, Y., & Lu, B. (2019). ATTEC: a potential new approach to target proteinopathies. *Autophagy*, *16*(1), 185–187. https://doi.org/10.1080/15548627.2019.1688556

Liao, X., Qin, G., Liu, Z., Ren, J., & Qu, X. (2023). Bioorthogonal Aptamer‐ATTEC conjugates for degradation of Alpha‐Synuclein via Autophagy‐Lysosomal pathway. *Small*, *20*(8). https://doi.org/10.1002/smll.202306760

Liu, Y., Sliter, D. A., Shammas, M. K., Huang, X., Wang, C., Calvelli, H., Maric, D. S., & Narendra, D. P. (2021). Mt-Keima detects PINK1-PRKN mitophagy in vivo with greater sensitivity than mito-QC. *Autophagy*, *17*(11), 3753–3762. https://doi.org/10.1080/15548627.2021.1896924

Liu, Z., Hu, M., Yang, Y., Du, C., Zhou, H., Liu, C., Chen, Y., Fan, L., Ma, H., Gong, Y., & Xie, Y. (2022). An overview of PROTACs: a promising drug discovery paradigm. *Molecular Biomedicine*, *3*(1). https://doi.org/10.1186/s43556-022-00112-0

Liu, Z., Qin, G., Yang, J., Wang, W., Zhang, W., Lu, B., Ren, J., & Qu, X. (2023). Targeting mitochondrial degradation by chimeric autophagy-tethering compounds. *Chemical Science*, *14*(40), 11192–11202. https://doi.org/10.1039/d3sc03600f

Marei, H., Tsai, W. K., Kee, Y., Ruiz, K., He, J., Cox, C., Sun, T., Penikalapati, S., Dwivedi, P., Choi, M., Kan, D., Saenz-Lopez, P., Dorighi, K., Zhang, P., Kschonsak, Y. T., Kljavin, N., Amin, D., Kim, I., Mancini, A. G., . . . De Sousa E Melo, F. (2022). Antibody targeting of E3 ubiquitin ligases for receptor degradation. *Nature*, *610*(7930), 182–189. https://doi.org/10.1038/s41586-022-05235-6

Mehra, S., Sahay, S., & Maji, S. K. (2019). α-Synuclein misfolding and aggregation: Implications in Parkinson's disease pathogenesis. *Biochimica Et Biophysica Acta (BBA) - Proteins and Proteomics*, *1867*(10), 890–908. https://doi.org/10.1016/j.bbapap.2019.03.001

Meléndez, A., & Levine, B. (n.d.). *Figure 1, Schematic diagram of the steps of autophagy. - WormBook - NCBI Bookshelf.* https://www.ncbi.nlm.nih.gov/books/NBK116074/figure/autophagy_figure1/

Menzies, F. M., Fleming, A., Caricasole, A., Bento, C. F., Andrews, S. P., Ashkenazi, A., Füllgrabe, J., Jackson, A., Sanchez, M. J., Karabiyik, C., Licitra, F., Ramirez, A. L., Pavel, M., Puri, C., Renna, M., Ricketts, T., Schlotawa, L., Vicinanza, M., Won, H., . . . Rubinsztein, D. C. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. *Neuron*, *93*(5), 1015–1034. https://doi.org/10.1016/j.neuron.2017.01.022

Mieland, A. O., Beyer, M., & Krämer, O. H. (2022). News and views. *Archives of Toxicology*, *96*(7), 2143–2144. https://doi.org/10.1007/s00204-022-03312-3

Mikitiuk, M., Barczyński, J., Bielski, P., Arciniega, M., Tyrcha, U., Hec, A., Lipińska, A. D., Rychłowski, M., Holak, T. A., & Sitar, T. (2023). IGF2 Peptide-Based LYTACs for targeted degradation of extracellular and transmembrane proteins. *Molecules*, *28*(22), 7519. https://doi.org/10.3390/molecules28227519

Nalawansha, D. A., & Crews, C. M. (2020). PROTACs: an emerging therapeutic modality in precision medicine. *Cell Chemical Biology*, *27*(8), 998–1014. https://doi.org/10.1016/j.chembiol.2020.07.020

News-Medical. (2019, March 27). *Limitations of Aptamers*. https://www.news-medical.net/life-sciences/Limitations-of-Aptamers.aspx

Pance, K., Gramespacher, J. A., Byrnes, J. R., Salangsang, F., Serrano, J. C., Cotton, A. D., Steri, V., & Wells, J. A. (2022). Modular cytokine receptor-targeting chimeras for targeted degradation of cell surface and extracellular proteins. *Nature Biotechnology*, *41*(2), 273–281. https://doi.org/10.1038/s41587-022-01456-2

Pangilinan, C., Klionsky, D. J., & Liang, C. (2024). Emerging dimensions of autophagy in melanoma. *Autophagy*, *20*(8), 1700–1711. https://doi.org/10.1080/15548627.2024.2330261

Paudel, R. R., Lu, D., Chowdhury, S. R., Monroy, E. Y., & Wang, J. (2022). Targeted protein degradation via lysosomes. *Biochemistry*, *62*(3), 564–579. https://doi.org/10.1021/acs.biochem.2c00310

Sakamoto, K. M., Kim, K. B., Kumagai, A., Mercurio, F., Crews, C. M., & Deshaies, R. J. (2001). Protacs: Chimeric molecules that target proteins to the Skp1–Cullin–F box complex for ubiquitination and degradation. *Proceedings of the National Academy of Sciences*, *98*(15), 8554–8559. https://doi.org/10.1073/pnas.141230798

Sarkar, S., Olsen, A. L., Sygnecka, K., Lohr, K. M., & Feany, M. B. (2021). α-synuclein impairs autophagosome maturation through abnormal actin stabilization. *PLoS Genetics*, *17*(2), e1009359. https://doi.org/10.1371/journal.pgen.1009359

Scholes, N. S., Mayor-Ruiz, C., & Winter, G. E. (2021). Identification and selectivity profiling of small-molecule degraders via multi-omics approaches. *Cell Chemical Biology*, *28*(7), 1048–1060. https://doi.org/10.1016/j.chembiol.2021.03.007

Schwalm, M. P., Dopfer, J., Kumar, A., Greco, F. A., Bauer, N., Löhr, F., Heering, J., Cano-Franco, S., Lechner, S., Hanke, T., Jaser, I., Morasch, V., Lenz, C., Fearon, D., Marples, P. G., Tomlinson, C. W. E., Brunello, L., Saxena, K., Adams, N. B. P., . . . Rogov, V. V. (2024). Critical assessment of LC3/GABARAP ligands used for degrader development and ligandability of LC3/GABARAP binding pockets. *Nature Communications*, *15*(1). https://doi.org/10.1038/s41467-024-54409-5

Stevens, C. M., Zhou, Y., Teng, P., Rault, L. N., Liao, Y., & Tang, W. (2023). Development of oligomeric mannose-6-phosphonate conjugates for targeted protein degradation. *ACS Medicinal Chemistry Letters*, *14*(6), 719–726. https://doi.org/10.1021/acsmedchemlett.2c00479

Summary of PROTAC degraders in clinical trials | Biopharma PEG. (n.d.). Retrieved June 19, 2025, from https://www.biochempeg.com/article/282.html?

Tabrizi, S. J., Ghosh, R., & Leavitt, B. R. (2019). Huntingtin Lowering strategies for disease modification in Huntington's disease. *Neuron*, *101*(5), 801–819. https://doi.org/10.1016/j.neuron.2019.01.039

Takahashi, D., Moriyama, J., Nakamura, T., Miki, E., Takahashi, E., Sato, A., Akaike, T., Itto-Nakama, K., & Arimoto, H. (2019). AUTACS: Cargo-Specific degraders using Selective Autophagy. *Molecular Cell*, *76*(5), 797-810.e10. https://doi.org/10.1016/j.molcel.2019.09.009

Takahashi, D., Ora, T., Sasaki, S., Ishii, N., Tanaka, T., Matsuda, T., Ikeda, M., Moriyama, J., Cho, N., Nara, H., Maezaki, H., Kamaura, M., Shimokawa, K., & Arimoto, H. (2023). Second-Generation AUTACs for Targeted Autophagic degradation. *Journal of Medicinal Chemistry*, *66*(17), 12342–12372. https://doi.org/10.1021/acs.jmedchem.3c00861

Takeshige, K., Baba, M., Tsuboi, S., Noda, T., & Ohsumi, Y. (1992). Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *The Journal of Cell Biology*, *119*(2), 301–311. https://doi.org/10.1083/jcb.119.2.301

Tan, S., Wang, D., Fu, Y., Zheng, H., Liu, Y., & Lu, B. (2023). Targeted clearance of mitochondria by an autophagy-tethering compound (ATTEC) and its potential therapeutic effects. *Science Bulletin*, *68*(23), 3013–3026. https://doi.org/10.1016/j.scib.2023.10.021

Tang, T., Liang, H., Wei, W., Han, Y., Cao, L., Cong, Z., Luo, S., Wang, H., & Zhou, M. (2023). Aloperine targets lysosomes to inhibit late autophagy and induces cell death through apoptosis and paraptosis in glioblastoma. *Molecular Biomedicine*, *4*(1). https://doi.org/10.1186/s43556-023-00155-x

Varshavsky, A. (2019). N-degron and C-degron pathways of protein degradation. *Proceedings of the National Academy of Sciences*, *116*(2), 358–366. https://doi.org/10.1073/pnas.1816596116

Vatté, J., Bourdeau, V., Ferbeyre, G., & Schmitzer, A. R. (2024). Gaining Insight into Mitochondrial Targeting: AUTAC-Biguanide as an Anticancer Agent. *Molecules*, *29*(16), 3773. https://doi.org/10.3390/molecules29163773

Wang, N., Cheng, X., Li, N., Wang, H., & Chen, H. (2018). Nanocarriers and their loading strategies. *Advanced Healthcare Materials*, *8*(6). https://doi.org/10.1002/adhm.201801002

Wang, Y., Yang, L., Yan, C., Du, Y., Li, T., Yang, W., Lei, L., He, B., Gao, H., Peppas, N. A., & Cao, J. (2024). Supramolecular artificial Nano-AUTACs enable tumor-specific metabolism protein degradation for synergistic immunotherapy. *Science Advances*, *10*(25). https://doi.org/10.1126/sciadv.adn8079

Wang, Z., Liu, C., Huang, S., & Chen, J. (2018). Wnt Signaling in vascular eye diseases. *Progress in Retinal and Eye Research*, *70*, 110–133. https://doi.org/10.1016/j.preteyeres.2018.11.008

Widemann, B. C., & Adamson, P. C. (2006). Understanding and managing methotrexate nephrotoxicity. *The Oncologist*, *11*(6), 694–703. https://doi.org/10.1634/theoncologist.11-6-694

Wilson, D. M., Cookson, M. R., Van Den Bosch, L., Zetterberg, H., Holtzman, D. M., & Dewachter, I. (2023). Hallmarks of neurodegenerative diseases. *Cell*, *186*(4), 693–714. https://doi.org/10.1016/j.cell.2022.12.032

Wu, P., Nielsen, T. E., & Clausen, M. H. (2015). FDA-approved small-molecule kinase inhibitors. *Trends in Pharmacological Sciences*, *36*(7), 422–439. https://doi.org/10.1016/j.tips.2015.04.005

Wu, Y., Lin, B., Lu, Y., Li, L., Deng, K., Zhang, S., Zhang, H., Yang, C., & Zhu, Z. (2023). Aptamer‐LYTACs for targeted degradation of extracellular and membrane proteins. *Angewandte Chemie International Edition*, *62*(15). https://doi.org/10.1002/anie.202218106

Zellner, S., Schifferer, M., & Behrends, C. (2021). Systematically defining selective autophagy receptor-specific cargo using autophagosome content profiling. *Molecular Cell*, *81*(6), 1337-1354.e8. https://doi.org/10.1016/j.molcel.2021.01.009

Zhang, D., Duque-Jimenez, J., Facchinetti, F., Brixi, G., Rhee, K., Feng, W. W., Jänne, P. A., & Zhou, X. (2024). Transferrin receptor targeting chimeras for membrane protein degradation. *Nature*. https://doi.org/10.1038/s41586-024-07947-3

Zhang, X., Liu, H., He, J., Ou, C., Donahue, T. C., Muthana, M. M., Su, L., & Wang, L. (2022). Site-Specific chemoenzymatic conjugation of High-Affinity M6P glycan ligands to antibodies for targeted protein degradation. *ACS Chemical Biology*, *17*(11), 3013–3023. https://doi.org/10.1021/acschembio.1c00751

Zheng, J., He, W., Li, J., Feng, X., Li, Y., Cheng, B., Zhou, Y., Li, M., Liu, K., Shao, X., Zhang, J., Li, H., Chen, L., & Fang, L. (2022). Bifunctional compounds as molecular degraders for Integrin-Facilitated targeted protein degradation. *Journal of the American Chemical Society*, *144*(48), 21831–21836. https://doi.org/10.1021/jacs.2c08367

Zhong, G., Chang, X., Xie, W., & Zhou, X. (2024). Targeted protein degradation: advances in drug discovery and clinical practice. *Signal Transduction and Targeted Therapy*, *9*(1). https://doi.org/10.1038/s41392-024-02004-x

Zhou, P., Zhang, S., Li, L., Zhang, R., Guo, G., Zhang, Y., Wang, R., Liu, M., Wang, Z., Zhao, H., Yang, G., Xie, S., & Ran, J. (2024). Targeted degradation of VEGF with bispecific aptamer-based LYTACs ameliorates pathological retinal angiogenesis. *Theranostics*, *14*(13), 4983–5000. https://doi.org/10.7150/thno.98467

Zhu, C., Wang, W., Wang, Y., Zhang, Y., & Li, J. (2023). Dendronized DNA chimeras harness scavenger receptors to degrade cell membrane proteins. *Angewandte Chemie International Edition*, *62*(13). https://doi.org/10.1002/anie.202300694

Zong, Y., Li, H., Liao, P., Chen, L., Pan, Y., Zheng, Y., Zhang, C., Liu, D., Zheng, M., & Gao, J. (2024). Mitochondrial dysfunction: mechanisms and advances in therapy. *Signal Transduction and Targeted Therapy*, *9*(1). https://doi.org/10.1038/s41392-024-01839-8