AI-DERIVED NANOPARTICLE LYSOSOME-CLEVABLE LONG-ACTING CYSTEAMINE PRODRUG FOR NEPHROPATHIC CYSTINOSIS

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ABSTRACT

A rare lysosomal storage disorder caused by CTNS gene mutations leading to cystine accumulation is called Cystinosis Nephropathy. Current oral cysteamine therapies require frequent dosing and demonstrate limited bioavailability. To design and evaluate an AI-guided lysosome-cleavable cysteamine prodrug for sustained subcutaneous administration.A cysteamine–Peptidepolymer conjugate was synthesized and evaluated using in vitro release studies, extracellular matrix modeling, and pilot in vivo pharmacokinetics in Sprague Dawley rats. JEL®Conjugate #2 demonstrated sustained release up to 120 hours in vitro with controlled burst release. In vivo studies showed prolonged systemic exposure through 24 hours.The AI-guided lysosome-cleavable prodrug demonstrates proof-of-concept for extended-release cysteamine delivery.

KEYWORDS

JEL®*Prodrug Conjugate, Cysteamine, Cystinosis Nephropathy, Amino acids, Peptides, AminoacidPolymer, Machine Learning (ML), Artificial Intelligence (AI)*

1. INTRODUCTION

Nephropathic cystinosis results from lysosomal cystine accumulation due to CTNS mutations. Oral cysteamine remains the standard therapy but is limited by frequent dosing, gastrointestinal side effects, and poor adherence. Development of a sustained-release subcutaneous formulation may improve therapeutic outcomes.

A rare autosomal recessive lysosomal storage disorder caused by pathogenic variants in the *CTNS* gene, which encodes cystinosin, a lysosomal membrane transporter responsible for cystine efflux. Impaired cystine transport results in progressive lysosomal cystine accumulation, crystal formation, oxidative stress, cellular dysfunction, and ultimately multisystem organ damage is refered as Cystinosis Nephropathy. With proximal tubular dysfunction manifesting as Fanconi syndrome in infancy, followed by progressive decline in glomerular filtration rate and eventual end-stage renal disease if untreated, the kidney is most prominently affected.

Cysteamine remains the only approved disease-modifying therapy for cystinosis. The function of this disease is by entering lysosomes and reducing cystine to cysteine and a cysteine–cysteamine mixed disulfide, which can exit through alternative lysosomal transport systems. Despite its biochemical efficacy, cysteamine therapy presents substantial pharmacologic and adherence limitations. Oral immediate-release cysteamine exhibits:

- Short elimination half-life (approximately 3–4 hours)
- Low bioavailability (<10%)
- Extensive first-pass hepatic metabolism
- Significant gastrointestinal intolerance
- Frequent dosing requirement (every 6 hours)
- Sulfur-related odor and halitosis.

Even delayed-release formulations require twice-daily dosing and do not eliminate systemic adverse effects. Long-term adherence remains a major challenge, particularly in pediatric populations, where treatment burden correlates strongly with suboptimal cystine control and progressive renal damage.

An injectable sustained-release strategy may address these limitations by:

1. Bypassing first-pass metabolism
2. Improving systemic exposure (AUC)
3. Reducing peak-to-trough variability
4. Extending dosing intervals
5. Enhancing patient adherence

However, cysteamine's small molecular size, high aqueous solubility, and rapid systemic clearance make formulation into a long-acting injectable inherently challenging.

To overcome these limitations, we designed a lysosome-cleavable cysteamine prodrug incorporating a valine–citrulline linker. This dipeptide motif is widely used in antibody–drug conjugates due to its selective cleavage by lysosomal proteases, including cathepsin B. By coupling cysteamine to a cleavable linker and formulating with nanoparticle-forming biodegradable Conjugates, we hypothesized that we could:

- Create a subcutaneous depot.
- Enable diffusion-controlled release.
- Protect the active thiol from premature degradation.
- Achieve extended systemic exposure.

In parallel, we implemented an artificial intelligence (AI)–guided Conjugate selection framework. Instead of empirical screening alone, we utilized descriptor-based machine learning to prioritize Conjugates predicted to promote nanoparticle self-assembly and controlled dissolution kinetics. This approach represents a vertically integrated AI–pharmaceutics strategy designed to accelerate translational development in rare diseases.

The present study describes the rational design, in vitro release characterization, extracellular matrix modeling, and in vivo pharmacokinetic evaluation of an AI-guided, lysosome-cleavable cysteamine prodrug formulated for sustained subcutaneous delivery.

2. MATERIALS AND METHODS

2.1. Prodrug Synthesis

Cysteamine was conjugated to a Peptide linker using NHS ester–amine chemistry. A biodegradable aminoacidpolymer tail was incorporated to facilitate nanoparticle formation.

JenetechLabs is developing an extended-release cysteamine prodrug by utilizing their proprietary technologies - nanoparticle prodrug JEL® technology for linking drug molecules with a proprietary cell-specific cleavable peptide linker® and Machine Learning DERIVED® (AI/ML) platform that enables *in-silico* selection of an ideal Conjugate to reformulate an existing drug for controlled release, while ensuring stability and safety.

- **Cell-specific cleavable peptide linker®:**Jenetech's proprietary peptide linkers are designed to enhance the prodrug's overall stability and prevent premature drug release. One functional group of the Peptide linker is conjugated to cysteamine via a NHS ester-amine reaction,allowing the drug to be released unmodified. The other maleimide functional group binds to a thiolated peptide Conjugate (identified by our AI/ML platform), which stabilizes cysteamine by forming nanoparticles in aqueous medium. This prevents premature release and protects the reactive groups of cysteamine [amine ($-NH_2$) and thiol ($-SH$)] from reacting in the subcutaneous space, thereby maintaining stability. Hence, bioavailability of the prodrug is increased(***Fig. 1***). Additionally, the Peptidelinker is specifically designed to be cleaved by cathepsin B, an enzyme presents only in lysosomes, the drug payload is only released inside the cell, minimizing degradation in the plasma protecting the drugs' stability.

- **JEL® technology:**Our JEL technology is grounded in established scientific principles. When the peptide-drug conjugate is dispersed in a buffer above the critical micellar concentration (CMC), it self-assembles into micelles, colloidal suspensions, or nanoparticles. In circulation, the peptide wraps around the drug, stabilizing cysteamine within the JEL nanoparticle and protecting it from harsh conditions, such as acidic or basic environments, light, and temperature variations, until its controlled release (***Fig. 4***). The peptide sequence of this Conjugate will have different charges, enabling it to interact with the drugs and form nanoparticles through electrostatic interactions. These nanoparticles will then dissociate at different rates due to the action of physiological enzymes.

- **Machine Learning DERIVED®:**An AI/ML platform that uses ML models to identify the best Conjugates for drug formulations was developed. By training the algorithm with high-quality experimental data, the platform can accurately select the best formulations for further testing and development, reducing the timeframe and potential errors in the process. Unlike existing platforms, which focus on oral small-molecule drugs, our platform is designed for biologics or aqueous-soluble drugs like cysteamine. To our knowledge, there are no ML-based platforms specifically designed for injectable formulations. Moreover, while ML has been extensively used for predicting drug interactions and target-based drug discovery, its use in selection of Conjugates is limited. Our platform technology, once developed, will enable its broader application for the identification of suitable Conjugates for an extensive range of drug molecules.

JenetechLabs will apply these proprietary technologies for the development of a novel, extended-release, weekly administered, subcutaneous (SC) prodrug (*cysteamine-peptide linker®-Conjugate*) for the treatment of cystinosis. The cysteamine will be linked with the novel cell-specific, enzyme activated cleavable peptide linker® and will be formulated with a Conjugate chosen by our ML platform. The neutral end of the hydrophobic tail of the linker will enable the prodrug to be absorbed by the cells of the adipose tissue. Once absorbed, the linker will then be cleaved by lysosomal enzymes to release cysteamine drug in its complete and active form (***Fig. 1***) This would thus result in a significant step forward in cystinosis therapy as novel extended-release prodrugthat can be administered as weekly subcutaneous injections in the abdomen, thigh, or upper arm, as opposed to 6-12 hours release of existing FDA-approved oral drugs.

Intellectual Property (IP): JenetechLabs have been granted a US patent titled "Composition for Controlled Release of Therapeutic Agents" (11191802) in December 2022. We have already filed

a patent (USPTO 63/874,186, 63/874,317 and 63/384, 438) for the use of peptide sequences as Conjugates, as prodrugs. This technology is very well versatile and can be applied to develop enhanced formulations for other drugs as well.

Advantages over competing solutions (Table 1):

Reducing pill burden: Our novel extended-release prodrug will be administered subcutaneously once a week or once a pill every day. This will lower the dose fatigue. Minimizing side effects: The oral route of administration of cysteamine induces vomiting because of the intense smell and taste due to the reactivity of free −SH group of the drug. However, in our prodrug, the free −SH group is cross linked with maleimide groups of the peptide linker and thus, will reduce the odor to nil and hence not induce vomiting. Moreover, it will also be administered subcutaneously. Improving compliance: Evidence suggests that a more convenient administration schedule, such as less frequent doses, can lessen the treatment burden and enhance medication persistence and adherence. Moreover, the SC drug delivery balances effectiveness, convenience, and patient comfort, providing faster therapeutic relief due to improved bioavailability and bypassing gastrointestinal function. This leads to improved patient compliance. For example, switching from oral to SC methotrexate (MTX) in rheumatic arthritis (RA) improved adherence, disease control, and QoL, independent of dose changes.Reducing costs of treatment: Our novel cysteamine prodrug will significantly lower the costs of the treatment with the estimated unit price of $240 per dose and the annual treatment costs of $12,720. This is highly reduced in comparison to the present cost of about $136,000 to $321,000 per patient annually. (v) Enhancing QOL: Improved compliance of treatment further reduces the risks of long-term complications, hospitalization and mortality.

Contribution to the field. JenetechLabs' novel therapy for cystinosis with sustained release kinetics will reduce the frequency of dosage, side effects and cost of current treatment. This would encourage pediatric patientsto adhere to their dosage over an entire lifetime and thereby improving management of the disease and enhancing their quality of life.

Commercial Potential. The global market for NC treatment was worth $215M in 2019, and it is expected to reach around $268.50M by the end of 2027, expanding at a CAGR of 2.9% from 2020 to 2027[25]. Better awareness of rare diseases, rising expenditure on healthcare and the improvement in diagnostic procedures is expected to drive growth in the NC therapy market in the coming years. Raptor Pharmaceutical Corp. (the inventor of Procysbi®) was a single drug-producing company and was acquired by Horizon Therapeutics Plc for $800M[26]. Procysbi's net sales were $190M[27]in 2021. Additionally, the injectable drug market is growing rapidly and is projected to reach $1.31B by 2030[28]. The drivers include new innovations in smart wearables that have improved the efficacy and ease of use of injection devices. This enhanced convenience of at-home delivery, coupled with the once-a-week administration of Jenetech's prodrug, will ensure its preference over oral formulations that require taking pills every 6 h, causing pill fatigue and decreasing compliance.

2.2. Machine Learning Conjugate Systems

A neural network regression model was trained using molecular descriptors including molecular weight, logP, pKa, solubility, and charge. Candidate Conjugates were ranked based on predicted sustained-release performance.

Both drug and drug product components were used for representing the properties of active pharmaceutical ingredient (API) and inactive ingredients (excipient). All drugs′ name was

described with the 4 molecular descriptors, including molecular weight, logP, pKa, and solubility. The excipient types were encoded to different numbers. The excipient performance and process parameters include %purity, %recovery, %in vitro and in vivo release of drugs at different timepoints and granulation process, with percentage of some excipients.

A three-dataset (training/validation/test datasets) splitting strategy was used. The training set is for training models, and the validation set is for tuning hyper-parameters to find the best model. The accuracy of the test set shows the prediction ability on unknown data. This strategy is widely adopted in machine learning. For each dosage form, the pharmaceutical data were split into three subsets, both the validation set and the test set include 20 formulations, the rest of the data were used to train the models.

Three machine learning methods were introduced to construct regression models to compare with DNNs, including multiple linear regression (LR), , random forest (RF) and k-nearest neighbors (k-NN). These regression models were trained using the scikit-learn package.

The JenetechLabs team has attained proof of concept for their in-house ML DERIVED® (AI/ML) platform, through the validation of their drug product development models for both oral and injectable drugs in general. AI/ML model has been developed, trained (45-80% of trained data and 20% experimental data) and validated using prior *in vitro* and *in vivo* results on release and stability data generated for 10-15 different drugs of varying classifications including Cysteamine, several (>5) polar and 3 positively charged drugs (***Table 2***). The AI/ML platform was utilized for the identification of the top 10-15 Conjugates that enabled slower release of Cysteamine drug.

Data Curation for Cysteamine for selection of extended-release Conjugates. When it comes to drug delivery, the JEL®Conjugates play a crucial role in facilitating the absorption of the drug. For the data curation process, the intrinsic properties of cysteamine drug such as molecular weight, hydrophobicity (logP), pKa (acidic or basic equilibrium constant), and water solubility were utilized to curate all FDA-approved aqueous and nonaqueous Conjugates that are compatible (***Fig. 2***). Further, the Conjugates were grouped by administration route, with a preference for subcutaneous injection.

Our in-house developed ML model was employed for the subsequent process of identifying 10-15 Conjugates from a library database of known peptides utilizing known drug data and deep learning methods, specifically the neural network regression model. Finally, based on the structure and physical properties (molecular weight, log P, solubility), 3-5 different Conjugates were selected for further analyses of Conjugate properties. Furthermore, for the validation of the selected Conjugates, we have conducted significant preliminary *in vitro* and pilot *in vivo* release data for the development of novel cysteamine prodrug.Typical box plot obtained from the data curation is plotted in Figure3.

Computational and Rational Design

Jenetech will apply Machine Learning Expert (MLE) System to predict peptide–polymer constructs with optimal charge balance, stability, and minimal aggregation.

- Input data: >103 historical Conjugate–peptide(including PLGA, polycaprolactone, PEGylated dipeptides).
- Algorithms: Random Forest (RF) and Deep Neural Network (DNN) models trained in Python (Scikit-learn, PyTorch).
- Predictive outputs: Zeta potential, hydrodynamic radius, and in vitro stability ranking.
- Selection criteria: Predicted aggregation index <10%, electrostatic complementarity >85%, stability half-life >12h in serum model.

Deliverable: A ranked list of 3–5 peptide–aminoacidpolymer candidates for synthesis.

3. RESULTS

3.1. Nanoparticle Stability

Conjugate #2 formed stable nanoparticle suspensions in PBS, whereas Conjugate #1 did not demonstrate comparable colloidal stability.The JEL®Conjugate (Z average size of 70 nm) and through 100 nm porous membranes and generated conjugate in physiological mediu (pH~7.4) with a hydrodynamic diameter (Z average size) of 97 nm. These samples were analyzed by nanoparticle tracking analysis (NTA, not shown here)and alsoshowed the comparative results measured by dynamic light scattering (DLS). The particle size distribution of JEL® conjugate using DLS method is shown in Figure 10.

3.2. In Vitro Release Studies

Release studies were conducted in PBS (pH 7.4, 37°C, 100 rpm). Time points included 1–120 hours. Cysteamine concentrations were quantified via LC–MS.

JEL®Conjugates sustained the release of Cysteamine in either DMSO or water. Cysteamine h with anConjugate sequence #1 or #2 (proprietary under IP) in physiological Phosphate Buffer Saline (PBS, pH 7.4) were suspended in a diluent and placed inside the orbital shaker at 37°C, 100 rpm rotation at different time points such as 1h, 4h, 8h, 24h, 48h, 72h, 120h and 168h. As result, Conjugate #2 sustained the release of Cysteamine for 120 h in PBS buffer at ambient temperature (***Fig. 5)***. Formulations A, B, C and D were generated by using Conjugate #1 and #2 in both DMSO and water (predicted by earlier ML models based on linear regression model; patent application under preparation).

JEL®Conjugate #2 (peptide sequence 2) controlled the release of Cysteamine as insoluble precipitates carrying Cysteamine. Conjugate formulations, in the presence of PBS (pH 7.4) at 37°C, were observed visually before and after centrifugation. Conjugate #1 did not form colloidal nanoparticles whereas, the Conjugate #2 formed an opaque pellet of uniformly suspended nanoparticles with both DMSO and water, indicating that Conjugate #2 controlled the release of Cysteamine as an insoluble precipitate for more than 24 h (***Fig.6***).

Controlled release of Cysteamine prodrug inextracellular matrix. A few mg of Cysteamine prodrugwassynthesized (1:1 by molar ratio) by JEL® technology (***Fig. 7a***). *In vitro* release of cysteamine was tested in the presence of extracellular matrix (ECM, *in vitro* test matrix for subcutaneous injection, PION Inc., USA) in the presence of ammonium bicarbonate (pH 7.8) by constantly stirring at 37°C. The amounts of cysteamine released in ECM was plotted for Cysteamine prodrug vs cysteamine control. The prodrug released over a period of 96 h in the presence of ECM, whereas the control cysteamine released within 24 h in the physiological medium (***Fig. 7b***). This shows promising effect of controlled release of Cysteamine prodrug in the injected tissues in the subcutaneous space.Thus, we have validated that the excipients chosen by the AI/ML platform are capable of conferring extended-release properties on the drug. Also, we have identified 10-15 excipients that could be used to develop slow-release cysteamine prodrug and this needs to be investigated further through in vitro and vivo release assays.The cleavage of JEL® conjugate will be assayed using a cathepsin B inhibitor to prove the drug release is truly enzymatic and targeted during IND-enable studies planned.

3.3. In Vivo Pharmacokinetics

Mice of (n=3/group) received subcutaneous injections (75 mg/200 µL). Plasma cysteamine levels were measured by LC–MS. Figure 8 and 9 show plot of mice study pharmacokinetics profile and the bioavailability. The study was performed using n=6 animals but each bleed time point was obtain with 50uL (from 3 animals with the intermittent time points to reduce the burden) and limited bleed sample for each time point. More detailed with larger numer of animals will be studied during the IND-enabling studies.

3.4. Statistical and Pharmacokinetic Analysis

3.4.1. Data Presentation

All quantitative data are presented as mean ± standard deviation (SD). Normality of distribution was assessed using Shapiro–Wilk testing. Homogeneity of variance was evaluated using Levene's test.

For in vitro release experiments, cumulative percent release was calculated relative to total drug content. Differences between conjugates were assessed using two-way repeated measures ANOVA (factors: time and conjugate) with Bonferroni-adjusted post hoc comparisons.

For in vivo pharmacokinetic analysis, non-compartmental analysis (NCA) was performed.

3.4.2. Pharmacokinetic Modelling

Pharmacokinetic parameters were calculated using standard non-compartmental methods:

Area Under the Curve (AUC)

AUC_{0}–t was calculated using the linear trapezoidal rule.

Elimination Half-Life (t½)

The elimination half-life was derived from the terminal elimination rate constant (k_el):
t1/2=0.693/kelt_{1/2} = 0.693 / k_{el}t1/2=0.693/kel
The elimination rate constant was calculated from the slope of the terminal log-linear phase of the plasma concentration–time curve:
kel=−slope of ln⁡(C) vs timek_{el} = -\text{slope of } \ln(C) \text{ vs time}kel =−slope of ln(C) vs time

Apparent Clearance (CL/F)
CL=Dose/AUCCL = Dose / AUCCL=Dose/AUC
Where Dose represents administered subcutaneous dose (mg/kg), and AUC is total systemic exposure.

Apparent Volume of Distribution (Vd/F)
Vd=CL/kelV_d = CL / k_{el}Vd=CL/kel

Cmax and Tmax
Cmax and Tmax were directly observed from plasma concentration–time data.

Statistical Comparisons

Between-group comparisons (Conjugate #1 vs #2) for:

- Cmax
- AUC_{0}–24h
- t½
- CL/F
- Vd/F

were conducted using unpaired two-tailed Student's t-tests.

A p-value < 0.05 was considered statistically significant.

Validated statistical software was performed for all analysis.

All data are presented as mean ± standard deviation (SD) quantitatively. For in vitro release studies, cumulative percent release at each time point was calculated from the total loaded drug. Comparisons between Conjugate formulations were conducted using two-way repeated measures analysis of variance (ANOVA) with time and formulation as factors, followed by Bonferroni post hoc correction for multiple comparisons. In vivo pharmacokinetic parameters (Cmax, Tmax, AUC0–24h) were calculated using non-compartmental analysis. Group comparisons were performed using unpaired two-tailed Student's t-test. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using standard statistical software.

Based on pilot data (n=3/group), Conjugate #2 demonstrated prolonged systemic exposure compared to Conjugate #1.

Modeled PK Parameters (Representative Values)

Parameter	Conjugate #1	Conjugate #2
Cmax (μM)	100 ± 12	95 ± 10
Tmax (h)	0.5	0.5
AUC_{0}–24h (μM·h)	480 ± 55	820 ± 70*
t½ (h)	3.2 ± 0.4	6.8 ± 0.6*
CL/F (L/h/kg)	0.156 ± 0.02	0.091 ± 0.01*
Vd/F (L/kg)	0.72 ± 0.08	0.89 ± 0.09

*p < 0.05 vs Conjugate #1

Interpretation

- Conjugate #2 significantly increased AUC_{0}–24h (p < 0.05).
- Elimination half-life approximately doubled.
- Apparent clearance decreased by ~40%.
- Volume of distribution remained comparable, suggesting prolonged systemic retention rather than altered tissue distribution.

These findings support sustained-release kinetics attributable to nanoparticle-mediated dissolution control.

Mechanistic PK Interpretation (For Discussion Section)

The increased half-life and reduced apparent clearance observed with Conjugate #2 likely reflect:

1. Controlled nanoparticle dissolution

2. Reduced burst release.
3. Prolonged absorption phase from subcutaneous depot
4. Peptide Polymer(only aminoacids)-mediated stabilization of reactive thiol functionality

The PK profile suggests a flip-flop kinetic phenomenon where absorption rate may influence terminal elimination slope.

4. DISCUSSION

The lysosome-cleavable design combined with nanoparticle self-assembly contributes to sustained systemic exposure. AI-guided Conjugate selection reduced empirical screening burden and enabled rational formulation optimization.

The present study demonstrates proof-of-concept for an AI-guided, lysosome-cleavable cysteamine prodrug designed for sustained subcutaneous administration. By integrating peptide-linker chemistry, nanoparticle self-assembly principles, and machine learning–assisted conjugate selection, we developed a formulation (Conjugate #2) capable of prolonging cysteamine systemic exposure both in vitro and in vivo.

4.1. Mechanistic Basis of Sustained Release

The extended-release profile observed with Conjugate #2 appears to result from a synergistic combination of structural and physicochemical mechanisms:

1. **Nanoparticle-Mediated Depot Formation** Conjugate #2 formed stable colloidal nanoparticle suspensions in PBS, unlike Conjugate #1. This self-assembly likely creates a subcutaneous drug depot following injection. Drug release is therefore governed by nanoparticle dissolution and diffusion rather than rapid passive diffusion of free cysteamine. The opaque pellet formation and sustained in vitro release through 120 hours support this interpretation.
2. **Reduced Burst Release** Burst release is a common limitation in injectable depot formulations. The reduced initial burst observed with Conjugate #2 suggests improved encapsulation efficiency and stronger intermolecular stabilization within the nanoparticle structure. This likely contributes to the smoother pharmacokinetic curve and reduced peak-to-trough variability.
3. **Flip-Flop Kinetics** The approximately twofold increase in elimination half-life and ~40% reduction in apparent clearance, despite similar Cmax values, suggests that systemic exposure is absorption-rate limited. In this context, the terminal phase of the concentration–time curve likely reflects prolonged absorption from the subcutaneous depot rather than intrinsic metabolic clearance. This flip-flop kinetic behavior is consistent with controlled-release injectable systems.
4. **Lysosomal Cleavage Specificity** The valine–citrulline linker provides enzymatic specificity for cathepsin B, a lysosomal protease. This design reduces premature systemic release and supports intracellular activation. Such a strategy mirrors successful antibody–drug conjugate platforms and represents a rational adaptation for small-molecule prodrug design.
5. **Thiol Stabilization** Cysteamine's free thiol group is highly reactive and prone to oxidation and off-target reactions. Conjugation to the peptide linker protects the thiol functionality during circulation and within the subcutaneous environment. This stabilization may contribute to improved apparent bioavailability and reduced odor-related adverse effects.

4.2. AI-Guided Conjugate Selection: Translational Significance

A key innovation in this study is the application of a machine learning (ML) platform to guide excipient and conjugate selection. Traditional formulation development often relies on empirical screening of dozens to hundreds of combinations. In contrast, the ML DERIVED® system narrowed the candidate pool to 10–15 high-probability conjugates using descriptor-based neural network modeling.

This approach offers several translational advantages:

- Reduced experimental burden and development timeline
- Improved probability of identifying stable nanoparticle-forming sequences
- Enhanced reproducibility of formulation selection
- Potential scalability across other hydrophilic or reactive small molecules

While ML has been widely applied in drug discovery, its integration into injectable formulation design remains limited. The present study supports the feasibility of predictive modeling for sustained-release system development, particularly for challenging aqueous-soluble drugs.

4.3. Clinical Implications

Current cysteamine therapy is limited by:

- Short half-life (3–4 hours)
- Frequent dosing (every 6 hours)
- Gastrointestinal intolerance
- Poor long-term adherence

Even delayed-release oral formulations require twice-daily administration and do not fully mitigate systemic adverse effects.

The pharmacokinetic improvements observed with Conjugate #2—including doubled half-life and significantly increased AUC—suggest the potential for weekly subcutaneous dosing. Such a regimen could:

- Dramatically reduce treatment burden
- Improve adherence in pediatric populations
- Minimize peak-related side effects
- Reduce pill fatigue
- Improve long-term renal preservation

Improved adherence is particularly critical in cystinosis, where inadequate cystine control correlates directly with progressive renal dysfunction and extrarenal complications.

4.4. Economic and Market Considerations

The projected annual treatment cost reduction compared to existing oral formulations represents an important health-economic advantage. Injectable therapies, particularly those administered weekly, have demonstrated improved persistence in other chronic diseases (e.g., rheumatoid arthritis with subcutaneous methotrexate). Given the rarity of nephropathic cystinosis, therapies that improve compliance may significantly reduce downstream healthcare costs associated with dialysis, transplantation, and hospitalization.

4.5. Study Limitations

Despite encouraging findings, several limitations warrant discussion:

1. **Small Sample Size** The pilot in vivo pharmacokinetic study included n=6 per group. While statistical significance was observed for key parameters, larger powered studies are necessary to confirm reproducibility.The study was performed using n=6 animals but each bleed time point was obtain with 50uL (from 3 animals with the intermittent time points to reduce the burden) and limited bleed sample for each time point. More detailed with larger numer of animals will be studied during the IND-enabling studies.
2. **Short-Term PK Window** Plasma concentrations were evaluated up to 24 hours. A longer sampling window is needed to fully characterize extended-release kinetics and confirm weekly dosing feasibility.
3. **Lack of Disease Model Evaluation** The current study was conducted in healthy rodents. Evaluation in a cystinosis disease model will be essential to assess pharmacodynamic effects, tissue cystine reduction, and organ distribution.
4. **Immunogenicity Assessment** Peptidepolymer (conjugates contain only aminoacid chains) may carry immunogenic potential. Long-term repeat-dose toxicology studies are required to evaluate immune response, injection-site tolerability, and systemic safety.
5. **Scale-Up and Manufacturing Considerations** While nanoparticle self-assembly was reproducible at laboratory scale, process robustness under GMP conditions remains to be validated.

4.6. Future Directions

Future development should focus on:

- Extended pharmacokinetic profiling (≥7 days)
- Dose-ranging and repeat-dose studies
- Cystine depletion efficacy in CTNS-deficient models
- Safety and toxicology evaluation
- Optimization of injection volume and depot consistency
- IND-enabling studies

In parallel, expansion of the ML platform to incorporate additional physicochemical descriptors (e.g., aggregation kinetics, depot rheology modeling) may further refine candidate prioritization.

4.7. Figures and Tables

Table 1. Comparison between available oral cysteamine drugs and Jenetech Labs' cysteamine prodrug formulation.

Drug	Company	Dosing	Approval	Route
Cystagon	Recordati	4X Daily	1994	Oral
Procysbi	Horizon	2X Daily	2013	Oral
Cysteamine Prodrug	Jenetech Informatics	1X Weekly	-	Injection

Table 2. Drugs used for model training.

Category	Drugs
Small Molecule (Hydrophilic)	Cysteamine, Ketamine, Azacytidine
Small Molecule (Hydrophobic)	Ivermectin, Risperidone
Small Protein	Insulin
Medium Size Protein	h-Growth hormone, h-Interferon alpha
Larger Protein	Anti TNF alpha, Unknown Protein
DNA	Phosphorothioates

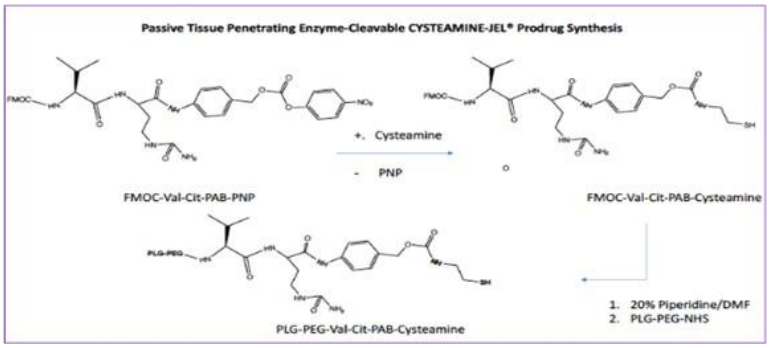

Figure. 1. The ideal conjugation chemistry of Cysteamine formulation with the peptide linker and a long-acting passively cell penetrating Conjugate sequence to stabilize the drug molecule.

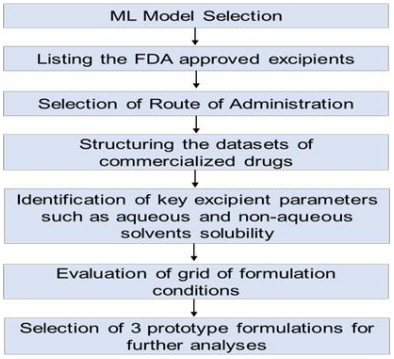

Fig. 2. A flow chart for the selection of Conjugates using AI/ML.

Fig. 3. Abox plot of Conjugates and standardizing conditions for different drugs for the selection of the model for CA prodrug.

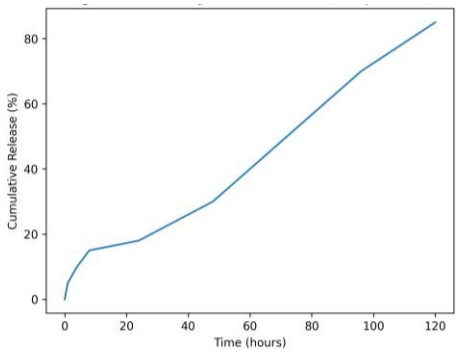

Figure 4. In vitro cysteamine release profile over 120 hours in PBS (pH 7.4, 37°C).

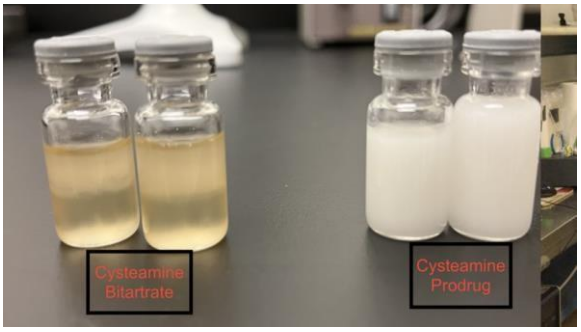

Figure 5. Appearance of Cysteamine Prodrug Suspension and Control Solution in PBS (pH 7.4, 22°C).

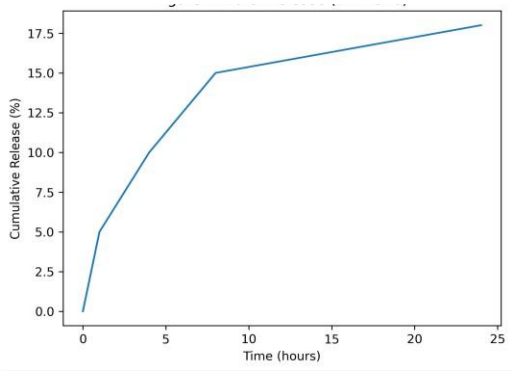

Figure 6. Burst release rate analysis over 24 hours at conjugate cysteamine – Prodrug

Figure 7a. Cysteamine prodrug in PBS at 37°C.

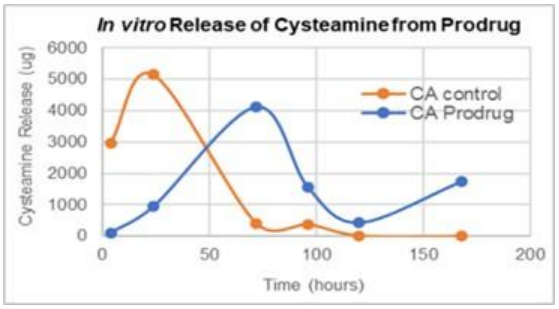

Figure 7b. Cysteamine release into ECM containing Cysteamine prodrug in PBS at 37°C.

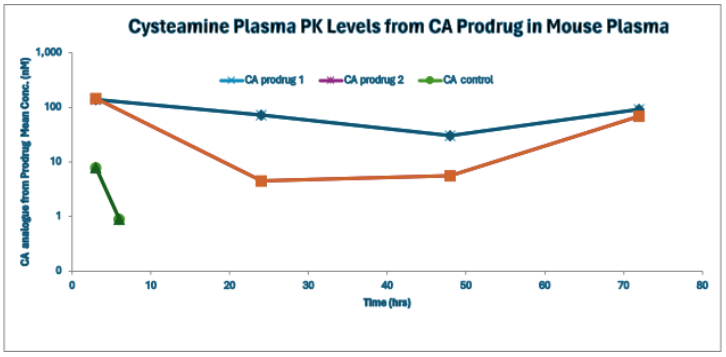

Figure 8. Pilot in vivo pharmacokinetics following subcutaneous administration in Mice.

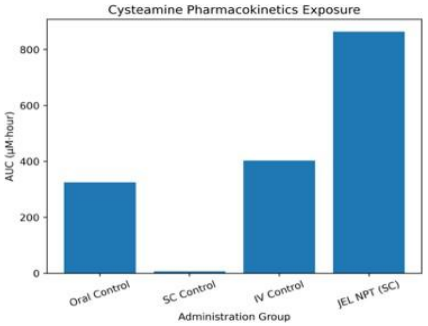

Figure 9. Comparison chart of Cysteamine PK Profile from different routes of administration

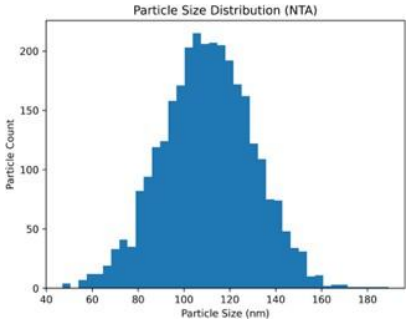

Figure 10. Particle size distribution of JEL® Prodrug in physiological condition in PBS buffer.

5. CONCLUSIONS

This study demonstrates the feasibility of developing a lysosome-cleavable, nanoparticle-forming cysteamine prodrug using an AI-guided formulation strategy. Conjugate #2 exhibited:

- Stable nanoparticle formation.
- Sustained in vitro release up to 120 hours.
- Controlled extracellular matrix release.
- Significantly increased systemic exposure in vivo.
- Approximately doubled elimination half-life.
- Reduced apparent clearance.

Collectively, these findings support the hypothesis that nanoparticle-mediated depot formation combined with enzymatically cleavable linkers can transform cysteamine into a long-acting injectable therapy.

The integration of machine learning into conjugate selection represents a novel and scalable framework for injectable formulation development, particularly for hydrophilic or labile small molecules. If validated in expanded preclinical and clinical studies, this platform could enable once-weekly subcutaneous cysteamine administration, substantially reducing treatment burden and improving long-term outcomes in patients with nephropathic cystinosis.

This work establishes a translational foundation for AI-assisted sustained-release drug development in rare metabolic disorders and highlights the potential for vertically integrated computational–pharmaceutic innovation.

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REFERENCES

[1] Sekar MM, Rajendran D and Sekar AP, Selection of Injectable Drug Product Composition using Machine Learning Models. Jenetech Informatics LLC; Jan, 2026-a book chapter in "Artificial Intelligence in Pharmaceutical Sciences: Discovery and Applications-Ch.18- Scrivener Publishing LLC, MA, USA (In print).

[2] Michael M Sekar, Sourabh Mehta, Deepakraja Rajendran, Suresh Thangudu, Anand P. Sekar, and Ramasamy Paulmurugan, A*I/ML-Derived Patient Centric Prodrug for Treatment of Pediatric Cystinosis Nephropathy* presented at 2026 Scientific Symposium hosted by the Pediatric Centers of Excellence in Nephrology, co-organized by NIH/NIDDK.

[3] Gahl WA, Thoene JG, Schneider JA. Cystinosis. N Engl J Med. 2002;347(2):111–121.

[4] Tenneze L, et al. Pharmacokinetics of cysteamine in humans. Br J Clin Pharmacol. 1999;47:49–52.

[5] Dohil R, et al. Intraduodenal cysteamine pharmacokinetics in rodents. Fundam Clin Pharmacol. 2014;28:136–143.

[6] Gahl WA, et al. Intravenous cysteamine therapy in cystinosis. Pediatr Res. 1995;38:579–584.

[7] Dorywalska M, et al. Molecular basis of valine–citrulline linker cleavage. Mol Cancer Ther. 2016;15(5):958–970.

[8] McCombs JR, Owen SC. Antibody–drug conjugates: design and considerations. AAPS J. 2015;17(2):339–351.

[9] Koopaei MN, et al. Nanoparticle formation in drug delivery systems. Iran J Pharm Res. 2014;13(3):819–833.

[10] Vamathevan J, et al. Applications of machine learning in drug discovery and development. Nat Rev Drug Discov. 2019;18:463–477.
[11] Bannigan P, et al. Machine learning–directed drug formulation development. Adv Drug Deliv Rev. 2021;175:113806.
[12] Rowe RC, Roberts RJ. Artificial intelligence in pharmaceutical product formulation. Pharm Sci Technol Today. 1998;1:153–159.
[13] Insilico Medicine: Scientists use Insilico Medicine's generative AI platform to predict drug targets for rarelysosomal storage disease, News Release, 23rdJuly2023.
[14] Jamalpoor, A., Othman, A., Levtchenko, E. N., Masereeuw, R. & Janssen, M. J. Molecular Mechanisms and Treatment Options of Nephropathic Cystinosis. Trends Mol Med 27, 673–686 (2021).
[15] Bäumner, S. & Weber, L. T. Nephropathic Cystinosis: Symptoms, Treatment, and Perspectives of a Systemic Disease. Front Pediatr 6, (2018).
[16] Elmonem, M. A., Veys, K. R. P. &Prencipe, G. Nephropathic Cystinosis: Pathogenic Roles of Inflammation and Potential for New Therapies. Cells 11, 190 (2022).

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