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Improved ApoptiCIDe™ Safety Switch Provides a Novel, Controllable Therapeutic Platform for Regulated Klotho Anti-Aging Gene Therapy

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Abstract

Background: Just as the increasing complexities of cell therapies has demonstrated the need for a rapid, safe and effective safety switch, novel gene therapies that extend past gene replacement for rare pediatric diseases would benefit from the same level of controllability. It remains difficult to control systemic levels of novel proteins produced by newer types of gene therapies simply by modifying gene therapy dose. Therefore, a regulatable gene therapy platform that could be used for such products would be advantageous. Currently, the rimiducid/inducible Caspase-9 (ApoptiCIDe™) system remains the most clinically validated attenuator for cell and gene therapy; however, it's broadest adoption is limited by the burden of a 2-hour infusion of the rimiducid dimerizer activating agent, along with infusion reactions that can occur as a result of the current excipient, Solutol hydroxy stearate (HS) 15. To improve efficacy and broaden potential applications of the ApoptiCIDe system, we developed PTC-1202, a reformulated rimiducid that can be administered quickly and easily *via* the subcutaneous or intramuscular route. Moreover, we demonstrated its broad utility *in vivo* by regulated attenuation of both reporter genes and therapeutically relevant anti-aging candidate therapeutics, Klotho (ApoptiCIDe-RGT-Klotho™ and Follistatin-344.

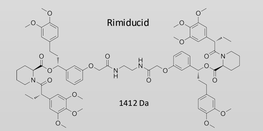
Results: Following a large excipient screen, we selected PTC-1202 for further clinical development with bioavailability above 30% via subQ and IM routes. Formal stability testing revealed 99.8% stability under accelerated conditions (40°C, 75% relative humidity) over 3 months. To demonstrate *in vivo* utility, bioluminescent (BLI) reporter, N-Luc, was co-expressed with ApoptiCIDe under the constitutive CMV promoter/enhancer and delivered to mice using AAV2 and AAV9 vectors via IV, IM, IP and SQ routes. In each case, administration of as little as 10 mg/kg rimiducid (0.3 mg total) led to ~ 90% elimination of BLI within days.

Regulated Gene Therapy: To demonstrate the utility of the **ApoptiCIDe-RGT™** regulatable gene therapy platform with a therapeutically useful gene target, we replaced N-Luc with human secreted Klotho (sKL1), associated with longevity or Follistatin (FST) 344, associated with maintaining muscle mass and delaying sarcopenia or overcoming muscular deficiencies. In both cases, levels of human proteins were easily detectable in the serum at physiological levels and elimination of protein(s) of up to 100% was achievable with one or more rimiducid administration. After a second injection, 60% of mice had undetectable human Klotho and the remaining animals revealed >90% decreased peak protein levels. Ongoing experiments are evaluating the use of lower doses of PTC-1202 as a "rheostat" to enable turning down rather than turning off the production of the therapeutic protein.

Regulated Cell Elimination: Furthermore, to demonstrate the versatility of the new activation ligand, PTC-1202, towards regulated cell elimination, as PoC, we developed a novel AAV9 vector, which triggers regulated elimination of white adipose tissue, primarily among visceral WATs, accounting for most of the health effects of accumulated WAT. A single injection of PTC-1202 was able to significantly reduce body weight and fat mass without a detectable drop in lean mass unlike semaglutide, which must be taken more frequently and results in loss of both fat mass and lean mass without other interventions.

Discussions: We demonstrated that our improved rimiducid formulation should have broad application in both cell and gene therapies, extending well beyond the clinically well-validated use of rimiducid in CAR-T and CAR-NK cell therapies. Furthermore, our controllable gene therapy platform may allow a broader and more economical conversion of expensive and frequently administered protein therapies to more long-lived, and less frequent viral or non-viral-based protein-production depots without compromising safety. In addition, we believe targeting pathological cell types, such as adipocytes for elimination using our clinical candidate vector, ApoptiCIDe-CE-WAT-001™, may provide a new rapid weight loss option, eliminating fat without affecting lean body or bone mass, for patients with morbid or sarcopenic obesity. Other undesirable cell types, such as hyperplastic or malignant prostate epithelial cells or senescent cells are also under investigation.

Introduction



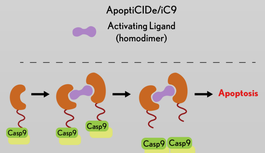
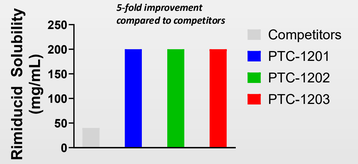


Figure: Interaction of the membrane-permeable, homodimeric activating ligand (rimiducid) with the enzymatically active domains of Caspase-9, modified to contain a ligand-binding domain, leads to enzymatic activation of Caspase-9 and rapid, non-inflammatory cell apoptosis. In ApoticIDe, to lower basal activity, the adapter Apaf-1-binding Caspase Activation & Recruitment Domain (CARD) has been replaced with rimiducid-binding FKBP12-F36V (Kd = 0.1 nM). PTC-1202 represents the re-formulated rimiducid.

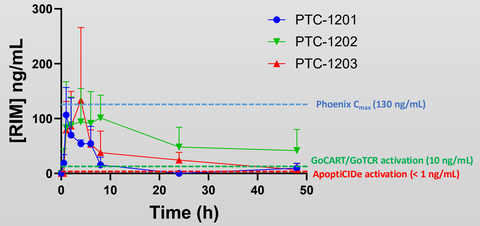
Results – Development of PTC-1202

- Rimiducid is often described as one of the least soluble molecules typically encountered by pharmacologists.
- Rimiducid was developed for the cell therapy safety switch, CaspaCIDe®, as a 40-mg infusion (5 mg/mL x 8 mL (25% Solutol HS15) → 100 mL saline), administered over 2 hrs with monitoring.
  - Cumbersome, costly, and the excipient is associated with infusion reactions
- Previous attempts by others to develop an oral or other formulation was hindered by its recalcitrant physical properties.
- To enable more widespread use, we set forth to develop a new, safer, more bioavailable, and easier to use formulation.

Solubility of Rimiducid in Excipients



PK Testing – Single intramuscular dose in dogs



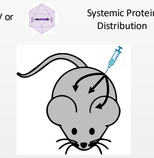
Stability Study

	PTC-1202			
	25°C/60% RH		40°C/75% RH	
Parameter	Assay (%)	Particle Diameter (nm)	Assay (%)	Particle Diameter (nm)
T = 0 month	99.9	54.2	100.1	54.2
T = 1 month	100.4	46.9	98.2	45.0
T = 2 month	102.0	42.3	102.4	41.7
T = 3 month	100.8	42.7	100.1	40.7

Figure: *Top Panel:* While rimiducid is only soluble in Solutol HS15 below 50 mg/mL, solubility increases to at least 200 mg/mL in excipients PTC-1201 to -1203. *Middle Panel:* Following a single intramuscular injection in dogs (0.02 mL/kg dose volume, 50 mg/mL rimiducid formulation, 1 mg/kg dose level), serum levels were determined by LC/MS analysis. This demonstrated easily achievable serum levels well in excess of levels needed to activate ApoptiCIDe. *Bottom Panel:* A 3-month standard stability study at RT and accelerated stability at 40°C demonstrated stable particle size without evidence of drug degradation.

Results – Regulated expression of secreted Klotho and Follistatin

ApoptiCIDe-RGT™-Regulated Protein Secretion



Examples: Mabs, anti-aging proteins, others

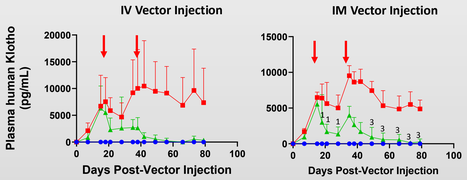


Figure: On day 0, cohorts of female 18-20-week-old C57Bl/6 mice (n = 5) received vehicle alone (blue circle), or a single IV or IM injection of AAV9 encoding a bicistronic vector encoding ApoptiCIDe-RGT-Klotho (CMV-driven ApoptiCIDe (rimiducid inducible Caspase-9)) along with secreted human a-Klotho (square and triangle). Vector-injected mice received PTC-1202 (reformulated rimiducid) on days 19 and 39 (triangle) or control excipient (square) and serum Klotho was analyzed by ELISA at the indicated timepoints. **Note:** Number shown represents the number of animals (of 5) with serum Klotho levels below the lower limit of quantification (LLoQ).

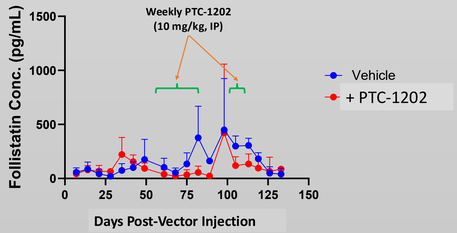
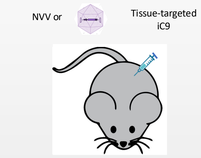
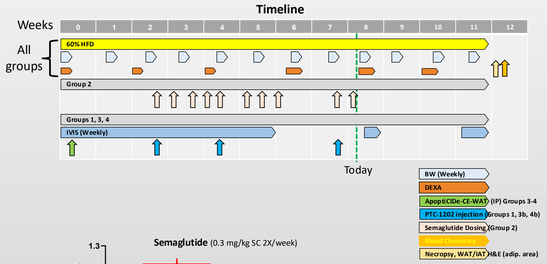


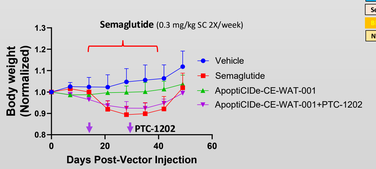
Figure: On day 0, cohorts of 7-8-week-old female Balb/c mice (n = 4) received a single IP injection of AAV2 (2.5E10 vg) encoding a bicistronic vector encoding ApoptiCIDe-RGT-Follistatin (CMV-driven ApoptiCIDe) along with secreted human Follistatin-344 (square and triangle). Vector-injected mice received drug formulation vehicle (blue circles) or PTC-1202 as shown (red circles) and serum follistatin was analyzed by ELISA at the indicated timepoints. **Note:** By Day 125, Follistatin levels were near background in both groups.

Results – Regulated elimination of white adipose tissue

ApoptiCIDe-CE™-Enabled Cell Elimination







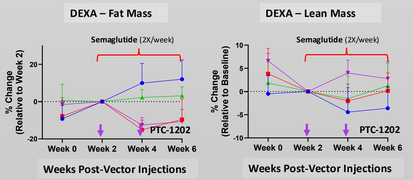


Figure: On day 0, two cohorts of 20-week-old, diet-induced obesity (DIO) C57BL/6 male mice (n = 3) were injected IP with (2.5E11 vg) AAV9 vector, ApoptiCIDe-CE-WAT, encoding ApoptiCIDe along with Nanoluciferase under a WAT – specific promoter (green triangles and purple triangles). Additional groups received saline (vehicle) only (negative control, blue circle) or GLP-1 agonist, semaglutide (positive control, red square). Mice were maintained on a 60% high-fat diet both before and during the study. Body weights were taken weekly and DEXA scan for body composition was performed biweekly. *Top Panel:* Prior to addition of semaglutide or activating ligand, PTC-1202, at day 14, weights of the vehicle control group and the semaglutide group were rising, but vector groups dropped slightly. Addition of semaglutide led to an abrupt drop in weight, nadiring at ~ 13% below peak, while addition of activating ligand led to a somewhat smaller drop of ~ 7%. While removal of semaglutide led to rapid weight gain within 1 week, weight loss by activating ligand was stable for 2 weeks before reversal. *Bottom Panels:* DEXA body scans demonstrated Fat loss by both semaglutide and PTC-1202, but loss of lean mass was only seen in the semaglutide group, consistent with clinical observations.

Conclusion and Next Steps

Rimiducid Reformulation: Next Steps

- Lead formulations established – Two injectable formulations show significantly higher bioavailability for ApoptiCIDe activation than necessary in dogs at clinically relevant dose volumes and doses
- Similarly, high bioavailability was seen by both IM and subcutaneous routes in mice and rats.
- Both of these formulations show good stability in a 3-month stability study.
- Pre-IND toxicity studies are pending (expected to start by 3Q2025)
- Scale-up, process development, analytical development, and other activities (outsourced to CDMO)
- Will commence with at least two lead formulations for redundancy in case issues arise with stability, processing, etc.
- Phoenix Senolytix is developed this reformulated rimiducid for use in ApoptiCIDe for purposeful cell elimination and as a safety- or rheo-switch for depot gene therapies.

Regulated expression of therapeutic proteins

- In the ApoptiCIDe-RGT setting, we demonstrated efficient (> 95%) elimination of both therapeutic proteins and Luciferase reporter (not shown) following vector-based delivered of ApoptiCIDe-enabled transgene and as few as 2 injections of PTC-1202.
- While we have focused here on the well-characterized aging-associated therapeutic candidates, Klotho and Follistatin-344, there are countless other candidate proteins, including pathogen-targeted Mabs or any other biologic that may require regulation in the event of unexpected toxicity or therapy obsolescence.
- Next steps underway include dose-finding studies, tissue-restriction of transgenes, phenotypic and toxicity studies.

Regulated elimination of White Adipose Tissue

- While GLP-1 agonists or incretin mimetics like semaglutide have had an amazing impact on reducing obesity worldwide, there effects are not limited to reducing fat mass, but loss of lean mass is also seen.
- Furthermore, there seems to plateau where further weight loss slows significantly or plateaus.
- In response, we developed a cell elimination switch based on targeting ApoptiCIDe primarily to visceral WAT.
- By removing a fraction of non-replaced white adipocytes in a regulated fashion, it may be possible to safely and non-invasively push beyond these plateaus.
- In addition to WAT, we are similarly investigating elimination of senescent cells and prostate cancer cells.

References and Contact Info

Key Rimiducid References

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- Steffin D et al. (2025) Interleukin-15-armoured GPC3 CAR T cells for patients with solid cancers. Nature 637, 940-6.

For more information, or if interested in licensing **ApoptiCIDe-RGT™**, contact: dspencer@Senotherapeutix.com or info@phoenixsenolytix.com