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ApoptiCIDe-RGT™ and ApoptiCIDe-CE™: Novel Regulated Cell and Gene Therapy Platforms Based on PTC-1202, a Proprietary, Injectable Rimiducid Formulation

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Abstract

Background: Inducible Caspase-9 (iCasp9)/CaspaCIDe™ remains the world's most utilized, clinically validated cell therapy safety switch, designed to be used only sporadically in case of a severe, uncontrolled cell therapy toxicity. It has been successfully utilized to control toxicities in allogeneic T-cell addback patients to control GvHD and in autologous CAR-T cell therapy-treated patients experiencing CRS and the difficult-to-manage ICANS at least 14 times in different settings by different investigators with a 100% success rate (FanL (99), DiStasiA (11), SteffinD (25), LocatelliF (25)). ApoptiCIDe™ is our re-imagined, proprietary version of CaspaCIDe, that utilizes the same iCaspase-9 rimiducid-triggered switch as CaspaCIDe but replaces Bellicum's, cumbersome and costly, intravenous rimiducid formulated in infusion reaction-associated Kolliphor HS15 with Phoenix's proprietary SC/IM rimiducid formulation, as its wholly owned small molecule dimer "trigger" for ApoptiCIDe.

Additionally, the rimiducid/FKBP12 system has been applied preclinically to countless other signaling switches and remains the most tractable inducible protein-protein interaction system with regards to ligand affinity and selectivity, immunogenicity and size of ligand-binding domain, off-target bioactivity of ligand, and research and clinical validation in cell and gene therapy. The lack of broad clinical availability and the challenge associated with of a prolonged, monitored infusion remains the main impediments to wider utilization. Moreover, the inclusion of a safety switch in cell therapies will become more essential as CAR-based cell therapies move beyond hematopoietic malignancies to more daunting solid tumor clinical settings that often require "armor" add-ons to increase potency, as well as into an *in vivo* format, in which both a titratable "rheostat" capability, in addition to a full "off" switch capability would enhance the utility and safety of this next generation "CAR T" platform. Finally, for dimerizer-based stimulatory switches that rely on repeated activating ligand administration, like the clinically validated, inducible MyD88-CD40 (iMC) "Go Switch", which enhances CAR-T cell efficacy, transitioning from an extended IV infusion to a quick and convenient injectable drug would be essential, particularly when cell elimination must be quickly and carefully titrated.

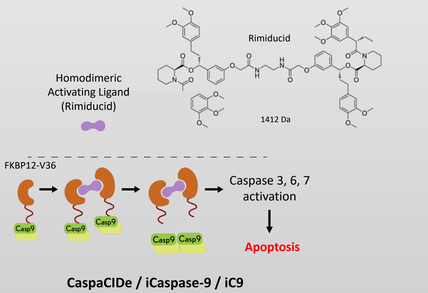
Methods & Results: Towards this goal, we converted the current 2-hour IV infusible formulation to a simple injectable formulation, PTC-1202, using GRAS reagents and have demonstrated excellent (99.8%) 3-M accelerated stability (40°C, 75% relative humidity) for this formulated ligand along with strong bioavailability in large mammals, easily reaching levels above 125 ng/mL in serum, orders of magnitude higher than the activation threshold for iCaspase-9.

To demonstrate the versatility of PTC-1202 towards regulated cell elimination, as PoC, we developed two novel gene therapy platforms, ApoptiCIDe-CD™, our purposeful cell elimination gene therapy platform, and ApoptiCIDe-RGT™, our regulated gene therapy platform. We initially developed the AAV9-based vector, ApoptiCIDe-CE-WAT-001™, which elicits regulated elimination of white adipose tissue (WAT) in the presence of rimiducid, especially within visceral WATs, a major site for the ill health effects associated with obesity, with undetectable activity in brown adipose tissue and non-fatty tissues. A single injection of PTC-1202 was able to significantly reduce body weight (BW) and fat mass without a detectable drop in lean mass, while weekly injections sustained the selective WAT cell loss. In contrast, the GLP-1 agonist, semaglutide (SMG), triggered a greater but less targeted initial BW loss that was followed by desensitization and BW relapse within a few weeks in the absence of dose escalation. Further, upon SMG removal, the whiplash weight gain was more profound than what occurred following PTC-1202 treatment termination. Moreover, in adult humans, the sustained effects of partial visceral fat removal should be more extensive, as adipogenesis is uncommon.

To demonstrate the versatility of PTC-1202 in broader settings, we titrated it into mice expressing a number of therapeutic proteins, including the pro-longevity protein, Klotho (sKL1) using novel vector ApoptiCIDe-RGT-Klotho™ and the use of vector ApoptiCIDe-RGT-MitoX-003™ to express one of our recently discovered gerotherapeutic peptides, MitoXcel™, which possess both senolytic and pro-mitochondria properties. In each case, PTC-1202 was able to reduce protein expression significantly, potentially to nearly undetectable levels, and protein levels could also be titrated to regulate expression level as a molecular "rheostat" function.

Discussions: Reformulated rimiducid, PTC-1202, opens up multiple new opportunities not readily achievable with the current rimiducid formulation, which remains for investigational use only. Here, we provide PoC for regulated and targeted cell elimination of WAT, but the ApoptiCIDe-CE platform should be adaptable for various other therapeutic targets including senescence and malignant cells. Moreover, the ApoptiCIDe-RGT platform should be useful for careful titration of many therapeutic proteins, relevant in longevity, oncology, immunotherapy and other applications.

Introduction



CaspaCIDe / iCaspase-9 / iC9

Figure Legend: 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.

Rimiducid/iCaspase-9 – Best in Class Safety Switch System

- 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% Kolliphor™ HS15) → 100 mL saline), administered over 2 hours with close monitoring.
  - This is cumbersome, costly, and the excipient is associated with infusion reactions (e.g., anaphylaxis).
- 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.

Successful use of rimiducid-activated iCaspase-9 for CRS/ICANS control in CAR-T cell therapy

Tumor indication	Target	Signaling	Dose	Tox	SoC	Rimid (mg/kg)	Time to recovery	CRR / #pts	Reference
r/r B-ALL-adult	CD19	4-1BB/z	1E6/kg	ICANS, Gr3-4	CS/Toci	0.4	12hr → Gr1, 4d → Gr0	1/1	Foster MC (21)
r/r B-ALL-adult	CD19	4-1BB/z	1E6/kg	ICANS Gr3-4	CS/Toci	0.1	12 – 24hr	3/3	G. Dotti, pers. comm.
NBM-ped	GD2	4-1BB/CD28/z	1E7/kg	ICANS, Gr3	Dex	0.4	< 24hr	4/4	Locatelli F (25)
Solid, mostly HCC, adult/ped	GPC3	4-1BB/z, IL15	3E7/m^2	CRS, Gr3	Toci / Anakinra	0.4	~ 4hr	3/3	Steffin D (25)
Solid, mostly HCC, adult/ped	GPC3	4-1BB/z, IL15	3E7/m^2	CRS, Gr3	Toci / Anakinra	0.004	< 24hr	3/3	D. Steffin, pers. Comm.
Resolution of Serious Toxicity				Total (known)					14/14

CS, corticosteroid; toci, tocilizumab; Dex, dexamethasone; ICANS, immune effector cell associated neurotoxicity syndrome; Gr, grade; r/r, relapsed and refractory; CRR, complete recover response; ALL, acute lymphoblastic leukemia; NBM, neuroblastoma; HCC, hepatocellular carcinoma

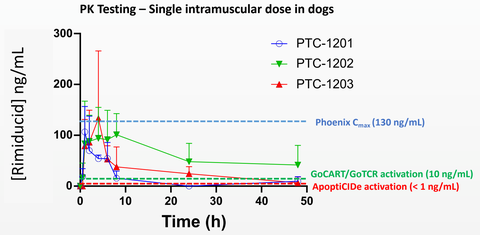
iCaspase-9: Best in Class Safety Switch Technology

Feature	ApoptiCIDe / iCaspase-9	RapaCasp9 / iRC9	EGFRt	ΔCD20 / RQR8	(Super Degron) SD-CAR	Switchable (CLBR001)	HSV-thymidine kinase (tk)
Activation Agent (Clinical availability)	Rimiducid	Rapamycin / Rapamune®	Cetuximab / Erbitux®	Rituximab / Rituxan®	Lenalidomide / analogs	SWI019 (others)	Valacyclovir
Completeness of elimination	Up to 99% (24h)	Up to 99% (48h)	partial, ADCC (leukocyte)-dependent	Up to 90%, ADCC (leukocyte)-depd.	90-95%	Probably complete	> 95%
Sensitivity ligand	< 1 nM	1-5 nM	High expr. needed	High expr. needed	~ 10 nM	NA	Yes
Speed of Action	Mins to Hrs	Mins to Hrs	Days	Days	Hours	2-3 days	Days
Bioactive ligand, Endogenous target	Rimiducid– no	Yes, Immune suppression	Yes, acneiform follicular skin exanthems, alb. Pain, nausea, asthenia	Yes, B-cell aplasia	Some toxicity at higher concentrations	No, Adapter	Yes/no, mutagenic
Immunogenicity	No	No	No	No	No	FITC-hapten?	Yes
Size protein/cDNA	~1.2-kb	~1.5-kb	~1-kb	~ 1-kb / 0.4-kb	+ 0.2-kb	NA	~ 1.2-kb
Clinical validation as gene therapy	Yes, 100%	No?	Frigault MJ (24) Blood; Lin H (24) Blood Cancer J	Griffioen M (09) Haem; Philip B (14) Blood	No	Nikolaenko L (21)-ASH Abstract	Bergler C (06) Gene Therapy
Reference(s)	FanL (99) Hum Gene Ther; DiStasiA11 NEJM	Stavrou M (18) Mol Ther			JanM (21) SciTransIMed		

NA, not applicable; depd. , dependent

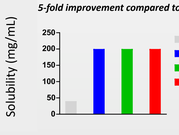
Key: Sufficient | Partial | insufficient

Results – Development of Injectable rimiducid, PTC-1202



Solubility of Rimiducid in Excipients

5-fold improvement compared to competitors



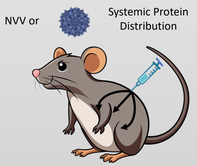
Stability Study

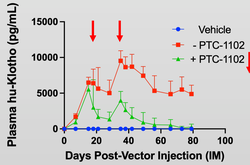
	PTC-1202			
	25°C/60% RH		40°C/75% RH	
Parameter	Assay (%)	Diameter (nm)	Assay (%)	Diameter (nm)
T = 0 M	99.9	54.2	100.1	54.2
T = 1 M	100.4	46.9	98.2	45.0
T = 2 M	102.0	42.3	102.4	41.7
T = 3 M	100.8	42.7	100.1	40.7

Figure Legend: *Top 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 exceeding levels needed to activate ApoptiCIDe. *Bottom Left 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 PTC-1203. Bottom *Right Panel:* A 3-month (M) 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

ApoptiCIDe-RGT™-Regulated Protein Secretion as a Gene Therapy (GT)

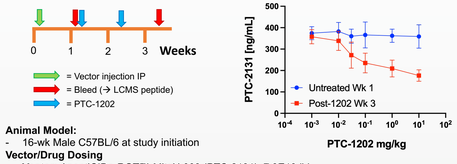




Examples: Mabs, anti-aging proteins, others

Figure Legend: On day 0, cohorts of female 18-20-week-old C57BL/6 mice (n = 5) received vehicle alone (blue circle), or a single IM injection of AAV9 (2.5E10) encoding a bicistronic vector expressing 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, injectable rimiducid) on days 19 and 39 (triangle) or control excipient (square) and serum Klotho was analyzed by ELISA at the indicated timepoints.

Regulated expression of MitoXcel™ geropeptide PTC-2131



Animal Model:
- 16-wk Male C57BL/6 at study initiation

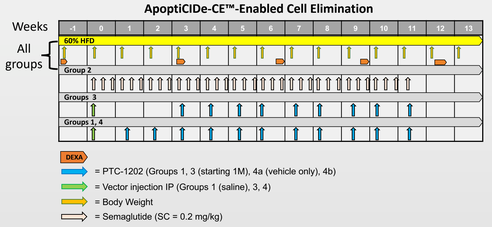
Vector/Drug Dosing
- **Vector:** ApoptiCIDe-RGT™-MitoX-003 (PTC-2131), 7.9E10 IV
- **Drug Titration:** 0.01 – 10 mg/kg (0.001 = no drug)

End Point
- Quantitation of plasma peptides via LC-MS/MS

Figure Legend: On day 0, cohorts of 16-wo, male C57BL/6 mice (n = 3) received a single IV injection of AAV9 (7.9E10) encoding a bicistronic vector expressing ApoptiCIDe-RGT-MitoX-003 (CMV-driven ApoptiCIDe (rimiducid-inducible Caspase-9)) along with secreted PTC-2131. After 7d, animals were bled, followed by SC administration of half-log or log dilutions PTC-1202. After 1 wk, animals received a 2nd dose of PTC-1202 at the same concentration as the 1st dose/cohort. One week after the 2nd PTC-1202 dose, animals were rebled and all plasma samples were sent for LC-MS/MS analysis (by Touchstone Bioscience). IC_{50} ~ 0.03 mg/kg. **Notes: (a)** Control, AAV-naive animals showed undetectable PTC-2131 peptide. **(b)** Using a transposon or other gene-editing approach, without stochastic, nuclear AAV uncoating, should allow for up to 100% protein inhibition.

Results – Regulated elimination of white adipose tissue (WAT)

ApoptiCIDe-CE™-Enabled Cell Elimination



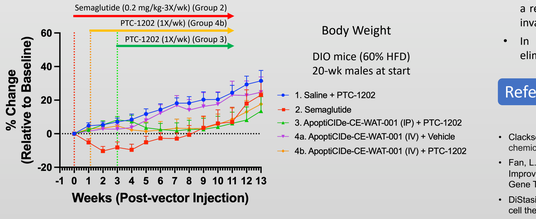


Figure Legend: On day 0, two cohorts of 20-week-old, diet-induced obesity (DIO) C57BL/6 male mice (n = 5) were injected IV with (2.5E11 vg) AAV9 vector, ApoptiCIDe-CE-WAT, encoding ApoptiCIDe along with Nanoluciferase under a WAT-specific promoter (purple triangles and orange diamonds). Alternatively, mice received AAV9 IP (green 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:* Timeline. *Bottom Panel:* Control (saline + excipient) mice steadily gained weight over 3 months, reaching 31.5% gain at week 13. Addition of semaglutide (d0) led to an abrupt drop in weight, nadiring at 10.3% below day 0 levels, while addition of activating ligand 8, or 21 days after vector addition prevented this weight gain in IV and IP-injected animals, respectively. While removal of semaglutide led to rapid weight gain within 1 week, weight loss by activating ligand was much more gradual, despite lipogenesis in mice. *Not Shown:* 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 experience with GLP-1 agonists.

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, plus both formulations show excellent stability in a 3-month stability study.
- Similarly, high bioavailability was seen by both IM and SC routes in mice and rats.
- Pre-IND toxicity studies are planned for 3Q2026
- Scale-up, process development, analytical development, and other activities (will be outsourced to CDMOs)
- Phoenix SENOLYTIX is developing this reformulated rimiducid for use in ApoptiCIDe for purposeful cell elimination and as a safety- or RheoSwitch for depot GT.

Regulated expression of therapeutic proteins

- In the ApoptiCIDe-RGT setting, we demonstrated efficient (> 95%) elimination of both therapeutic proteins (e.g., Klotho and MitoXcel) and luciferase reporter (not shown) following vector-based delivery of ApoptiCIDe-enabled transgene and as few as two PTC-1202 injections.
- This paradigm should apply to 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.
- Dose range-finding studies, tissue-restriction of transgenes, phenotypic and toxicity studies are underway.

Regulated elimination of White Adipose Tissue

- While GLP-1 agonists or incretin mimetics like SMG have had an amazing impact on reducing obesity worldwide, deleterious sarcopenia is also seen.
- Furthermore, weight loss can plateau or reverse following therapy termination or the absence of dose escalation.
- 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

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