DEMONSTRATION OF SYSTEMIC ANTITUMOR IMMUNITY VIA INTRATUMORAL REGULATED EXPRESSION OF IL-12 AS A GENE THERAPY APPROACH TO TREATMENT OF CANCER

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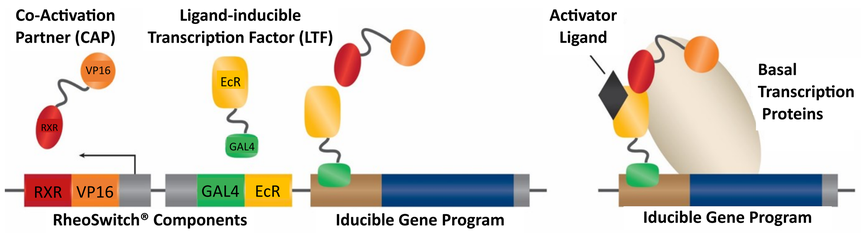
IL-12 and Cancer Immunotherapy

- Interleukin-12 (IL-12)
  - Pro-inflammatory cytokine
  - Master regulator of cell-mediated immunity to pathogens and neoplastic transformation
  - Produced by innate immune cells in response to pathogens
  - Leads to production by T- and natural killer (NK) cells of
    - Interferon gamma (INFγ)
    - Tumor necrosis factor alpha (TNFα)
  - Studies confirmed significant systemic toxicities
    - Prompted investigation of alternative delivery routes, e.g., sub-cutaneous, intratumoral, etc.
- Ad-RTS-IL-12 + veledimex explores regulated local treatment strategy
  - Goal is extending the IL-12 therapeutic window
  - Reducing systemic toxicity
  - Ad-RTS-IL-12 injected into tumor
  - IL-12 transcription up-regulated only in presence of activator ligand veledimex
  - IL-12 expression level can be modulated by dose and frequency of veledimex administration

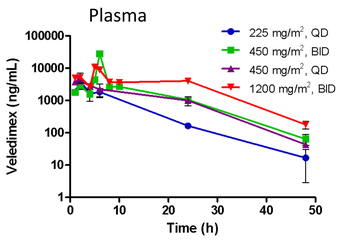
Inducible Gene Regulation: RheoSwitch Therapeutic System®

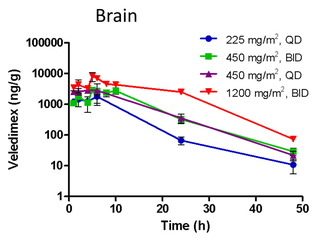
RheoSwitch Therapeutic System® (RTS®) is a 3-component transcriptional regulator

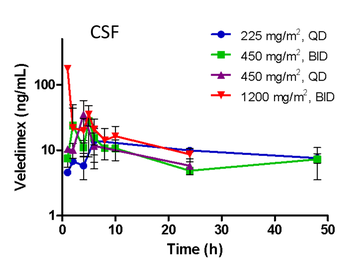


1. **The Switch Components**: The RTS® gene program includes 2 receptor protein fusions: VP16-RXR (co-activation partner, CAP) and Gal4-EcR (ligand-inducible transcription factor, LTF). In the absence of ligand, the LTF binds to the inducible promoter and does not form a stable interaction with the CAP.
2. **The Inducible Promoter**: A customizable promoter to which basal transcription proteins are recruited and the target gene is transcribed.
3. **The Activator Ligand (veledimex):** An ecdysone analog, diacylhydrazine-based small molecule functions as an activator. In the presence of the ligand, a conformational change in the LTF leads to a stable, high affinity interaction with the CAP.

Veledimex is Orally Absorbed & Crosses the Blood Brain Barrier





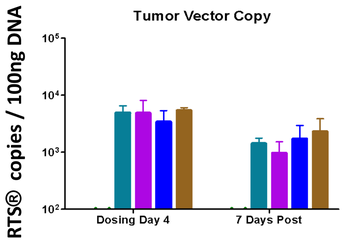


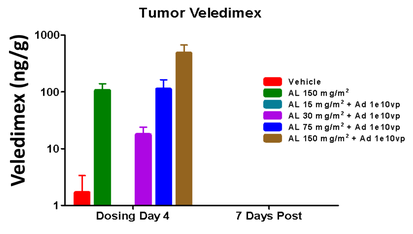
Veledimex was administered orally either as a single dose at 225 and 450 mg/m^2/day to C57BL/6 mice or as BID doses at 450 and 1200 mg/m^2/day with 4h between doses. Terminal plasma, CSF, and brain samples were collected from each animal at 1, 2, 4, 5 (BID only), 6, 8 (BID only), 10 (BID only), 24, and 48 h post dose. Samples were processed and analysed for veledimex using LC-MS/MS.

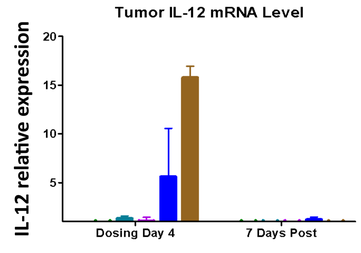
Summary of Veledimex Plasma, Brain & CSF Pharmacokinetics

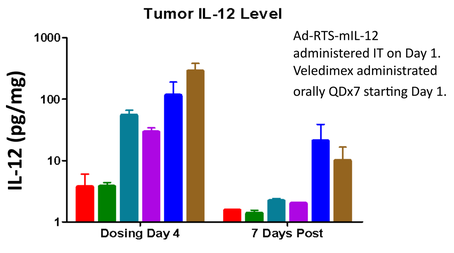
Plasma										
Dose	$t_{1/2}$	T_{max}	C_{max}	DN C_{max}	AUC_{0-t}	DN AUC_{0-t}	$AUC_{0-\infty}$	Vd/F	Cl/F	MRT_{0-t}
(mg/m^2/day)	(h)	(h)	(ng/mL)	(m^2*ng/mL/mg)	(h*ng/mL)	(m^2*h*ng/mL/mg)	(h*ng/mL)	(mL/kg)	(mL/h/kg)	(h)
225, QD	6.4	2	4153	18.5	33264	148	33418	20730	2244	6.9
450, QD	7.5	2	4057	9.02	59339	132	59810	27216	2508	11.8
450, BID	6.9	6	27733	61.6	101883	226	102525	14606	1463	10.5
1200, BID	8.7	5	10763	8.97	156599	130	158852	31704	2518	15.8
Brain										
Dose	T_{max}	C_{max}	DN C_{max}	%C_{max}	AUC_{0-t}	DN AUC_{0-t}	%AUC_{0-t}			MRT_{0-t}
(mg/m^2/day)	(h)	(ng/g)	(m^2*ng/g/mg)	Brain/plasma	(h*ng/g)	(m^2*h*ng/g/mg)	Brain/plasma			(h)
225, QD	6	1794	8.0	43.2	25325	112.6	76.1			6.43
450, QD	4	2810	6.2	69.3	47863	106.4	80.7			8.23
450, BID	5	2927	6.5	10.6	45613	101.4	44.8			10.4
1200, BID	5	8180	6.8	76.0	125393	104.5	80.1			13.86
CSF										
Dose	T_{max}	C_{max}	DN C_{max}	%C_{max}	AUC_{0-t}	DN AUC_{0-t}	%AUC_{0-t}			MRT_{0-t}
(mg/m^2/day)	(h)	(ng/mL)	(m^2*ng/mL/mg)	CSF/plasma	(h*ng/mL)	(m^2*h*ng/mL/mg)	CSF/plasma			(h)
225, QD	6	14	0.06	0.34	466.4	2.07	1.40			22.09
450, QD	4	35.2	0.08	0.87	265.2	0.59	0.45			8.59
450, BID	5	28.3	0.06	0.10	398.6	0.89	0.39			19.76
1200, BID	1	177	0.15	1.64	527.8	0.44	0.34			7.07

Dose-Dependent Increase in Expression of Tumor IL-12 mRNA & Protein in Response to Ad-RTS-mIL-12 (AD) + Veledimex in the 4T1 Syngeneic Mouse

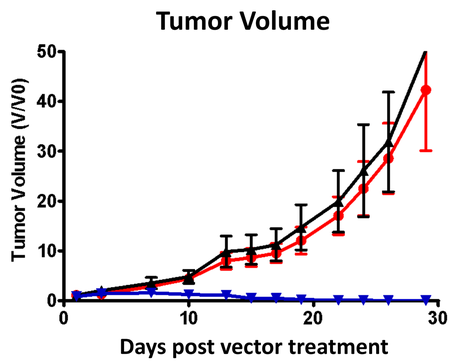


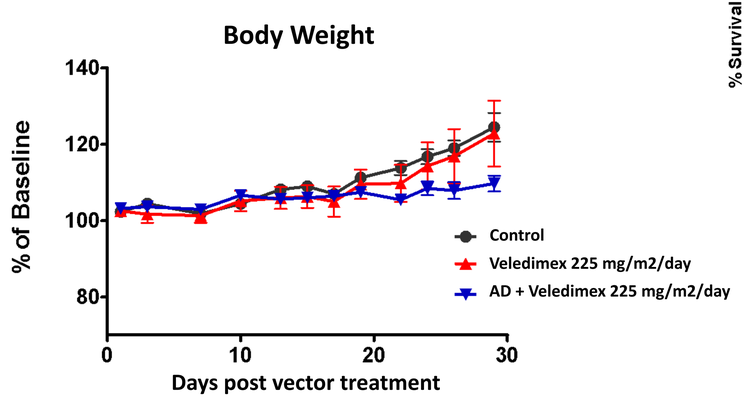






Ad-RTS-mIL-12 + Veledimex Induces Sustained Reduction in Tumor Growth in CT26 Syngeneic Mouse Colon Cancer Model

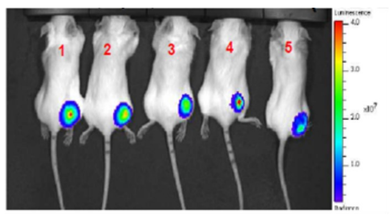




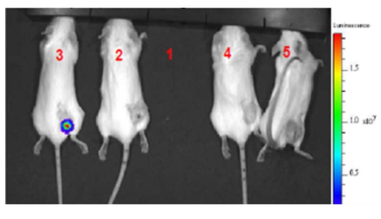
BALB/c mice were s.c. inoculated with 5x10^5 CT26 cells in the right flank. On Day 12, when tumors reached 50-100mm^3, animals were randomized to one of the treatment groups. Ad-RTS-mIL-12 was administered intratumoral at 1x10^{10}vp with veledimex p.o. administered continuously for the duration of the study. Tumor volumes were measured using LxW^2/2 and normalized to the individuals starting volume. Arrows depict Ad-RTS-mIL-12 treatment.

Ad-RTS-mIL-12 + Veledimex Induces Systemic Tumor Response in CT26-LUC Syngeneic Mouse Colon Cancer Model

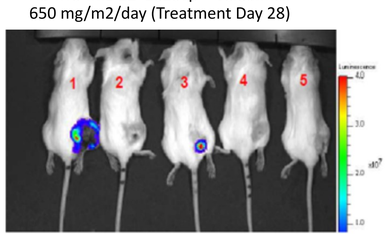
Baseline (15 days post CT26-LUC 1e5 cells)



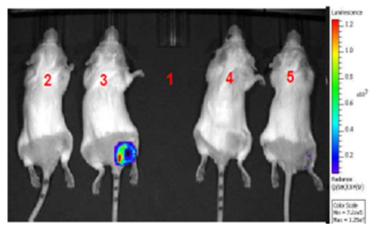
Treatment Day 33 rechallenged CT26-LUC Left Flank



Ad-RTS-mIL-12 1e10vp + Veledimex 650 mg/m2/day (Treatment Day 28)

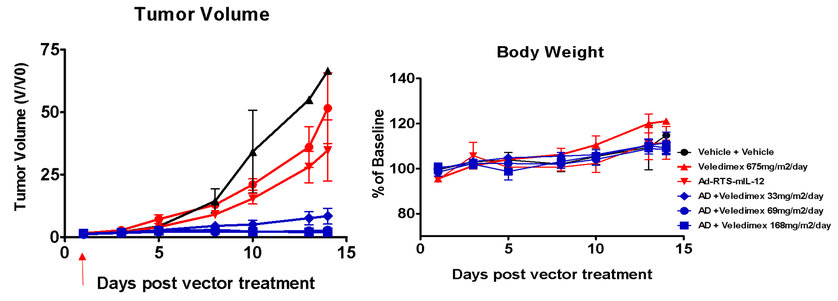


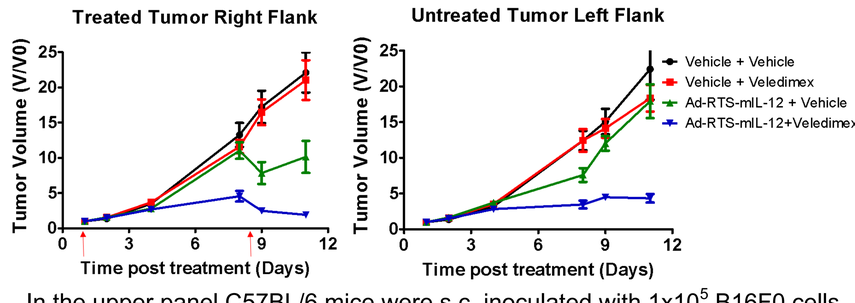
No Tumor Growth 21 Days post rechallenge



BALB/c mice were s.c. inoculated with 5x10^5 CT26LUC cells in the right flank. On Day 12, when tumors reached 50-100mm^3, animals were randomized to one of the treatment groups. Ad-RTS-mIL-12 was administered intratumoral at 1x10^{10}vp with veledimex p.o. administered continuously for the duration of the study. Tumor volumes were measured using LxW^2/2 and normalized to the individuals starting volume. Arrows depict Ad-RTS-mIL-12 treatment. In the second study animals were rechallenged with 5x10^5 CT26LUC cells in the left flank and monitored for an additional 21 days.

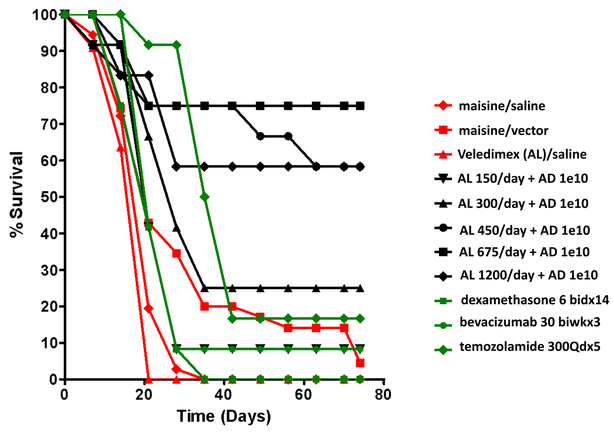
Ad-RTS-mIL-12 + Veledimex Induces Sustained Reduction in Tumor Growth in B16 Syngeneic Mouse Melanoma Model





In the upper panel C57BL/6 mice were s.c. inoculated with 1x10^5 B16F0 cells in the right flank. On Day 12, when tumors reached 50-100mm^3, animals were randomized to one of the treatment groups. A single dose of Ad-RTS-mIL-12 was administered intratumoral at 1x10^{10}vp with veledimex p.o. administered continuously beginning Day 1 for the duration of the study. Tumor volumes were measured using LxW^2/2 and normalized to the individuals starting volume. In the lower panel 1x10^5 B16F0 cells were inoculated s.c. in both the right and left flanks but only the right flank tumor being administered the vector. Arrows depict administration of Ad-RTS-mIL-12.

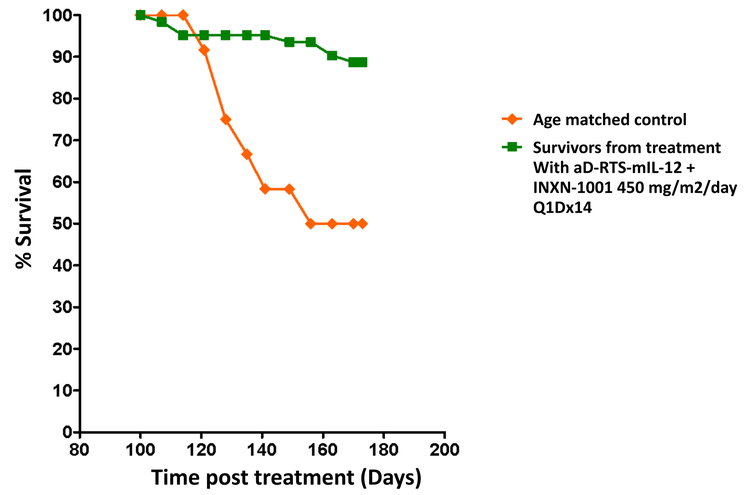
Ad-RTS-mIL-12 + Veledimex Elicits a Dose-Related Increase in Survival in the GL261 Orthotopic Glioma Mouse Model



Protocol

- Five Days prior to therapy 1 x 10^5 GL-261 glioma cells in 3 µl were administered into the brain of C57BL/6 mice.
- On Day 1 a single dose of Ad-RTS-mIL-12 at 1x10^{10}vp in 5 µl was administrated IC, followed by the activator ligand (veledimex) administered orally for 14 consecutive days. Standards of care were administered at the dose and schedule depicted.
- The time to disease progression and death was studied.

Ad-RTS-mIL-12 + Veledimex Pretreatment Induces Systemic Tumor Response in GL-261 Orthotopic Mouse Glioma Model



On Day 100 surviving animals administered Ad-RTS-mIL-12 1x10^{10}vp + veledimex 450 mg/m^2/day QDx14 were rechallenged with 1x10^5 GL-261 cells at the site of original implantation vs. age-matched controls administered 1x10^5 GL-261 cells .

Conclusions

- **Veledimex exhibited dose-related increases in plasma and brain tissue exposure.**
- **The increase in tumor veledimex levels in combination with Ad-RTS-mIL-12 resulted in an increase in expression of IL-12 mRNA leading to an increase in tumor IL-12p70 expression with minimal increase in serum IL-12.**
- **Dose-response was established, the optimal dose of orally administered veledimex was 450 mg/m^2 total dose + Ad-RTS-mIL-12 at 1x10^{10} vp.**
- **Ad-RTS-mIL-12 1x10^{10}vp + veledimex p.o. improved survival over temozolomide, dexamethasone and bevacizumab in an orthotopic glioma mouse model.**
- **Ad-RTS-mIL-12 + veledimex demonstrated systemic memory upon rechallenge in multiple syngeneic mouse models.**
- **These findings support the utility of localized regulatable IL-12 production as an approach for the treatment of malignant glioma.**