Treatment of Glioblastoma Through the Controlled Localized Production of IL-12 by the RheoSwitch Therapeutic System® Platform

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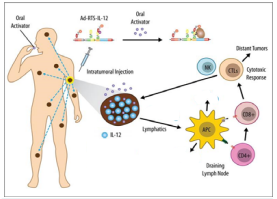
Abstract

Challenges in developing immunotherapies against glioblastoma include the immune-privileged status of the CNS and the physiological processes that contribute to the suppression of immune responses in the brain. The localized controlled production of IL-12 in the tumor may increase cytotoxic T cell infiltrate and subsequently reduce tumor vascularity and tumor volume, resulting in a prolongation of survival. We have developed an adenoviral vector, Ad-RTS-mIL-12 (AD), administered intratumorally under the control of the RheoSwitch Therapeutic System® (RTS®) expression platform. Gene expression and subsequent IL-12 protein production is controlled by the oral administration of the small molecule activator ligand veledimex (AL). We have shown the safety and biologic activity of this system in both nonclinical and clinical studies in melanoma and breast cancer. Based upon these favorable results we chose to study the activity of RTS-mIL-12 delivered intratumorally by an adenoviral vector-based approach in an orthotopic murine glioma model. The ability of AL to cross the blood brain barrier was assessed in both naive and orthotopic GL-261 tumor bearing C57B6 mice. The results in mice demonstrated that AL brain tissue levels increased from 25325 to 47868 ng*h/g in naïve mice at doses of 225-450 mg/m² p.o. administered on either QD or BID schedules while, as anticipated, the levels of AL in brain tissue increased in the tumor bearing mice. At 450 mg/m²/day BID p.o. increased brain tissue AL levels in GL261 mice increased by approximately 6 fold from 324±51 ng/g in normal mice to 1950±573 ng/g at Day 3 and were sustained through 14 days of continuous dosing (1150±212 ng/g). Based on these findings we assessed the effects of AD + AL on survival in the orthotopic GL261 syngeneic mouse glioma model where each animal received 1 x 10⁵ GL261 glioma cells via intracranial injection on Day 0. In this study AD was administered intratumorally at 1 x 10^{10} vp/animal on Day 5. AL was administered via gavage at doses of 450-1200 mg/m²/day for 14 consecutive days on a BID schedule. In addition, bevacizumab 30mg/m² biwkx3 and dexamethasone 6mg/m²/day BID x14 days were studied. AD + AL demonstrated a dose-related increase in survival benefit without exhibiting an adverse safety profile. At Day 100 with (study termination), 45, 50 and 68% of the animals that received doses of 450, 900 or 1200mg/m²/day, respectively, were alive. In contrast, the median survival in the vehicle control groups was 18 days, while in the bevacizumab and dexamethasone groups the median survival was 22 and 24 days, respectively. Additional studies in this model are ongoing to determine the optimal dose and schedule.
This novel regulated immunotherapeutic approach could potentially be translated into an effective clinical regimen for the treatment of glioblastoma.

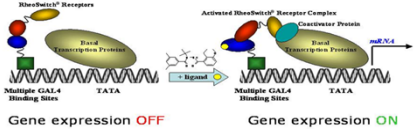
Background



Regulated intratumoral expression of IL-12 promotes activation of tumor-infiltrating lymphocytes to drive a cytotoxic immune response against distant tumors

- An adenoviral vector engineered to express IL-12 (Ad-RTS-hIL-12) utilizing the RheoSwitch Therapeutic System® (RTS®) technology is injected intratumorally
- Expression of IL-12 is controlled through the administration of an oral activator ligand (veledimex)
- Localized production of IL-12 leading to enhanced antigen presenting cells activity and T cell activation toward tumor-associated antigens, locally and systemically
- Influx of cytotoxic $CD8^+$ T cells coupled with a reduction in $CD4^+$ regulatory T cells

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

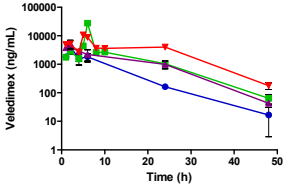


Gene expression OFF Gene expression ON

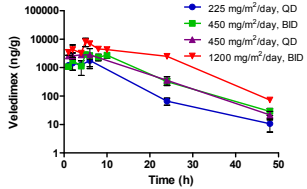
1. **The Switch Components**: The RTS® gene program includes two receptor protein fusions: VP16-RXR and Gal4-EcR. They form unstable and unproductive heterodimers in the absence of any ligand.
2. **The Activator Ligand:** An ecdysone analog, diacylhydrazine-based small molecule functions as an activator. In the presence of the ligand, the protein heterodimer changes to a stable conformation and binds to the inducible promoter.
3. **The Inducible Promoter**: A customizable promoter to which basal transcription proteins are recruited and the target gene is transcribed.

Results (Pharmacokinetics)

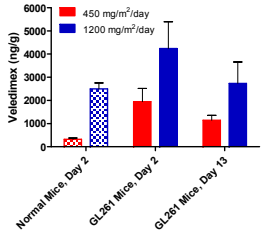
A. Dose-dependent Increase in Plasma Exposure of Veledimex (AL) in C57BL/6 Mice



B. Dose-dependent Increase in Brain Exposure of Veledimex (AL) in C57BL/6 Mice



C. Higher Veledimex (AL) Levels at 24 h in Brains of GL261 Orthotopic Glioma Mouse Model Than C57BL/6 Mouse



Assessment of the ability of veledimex (AL) to cross the blood brain barrier (BBB) in normal C57B/6 mice with intact BBB and in GL261 orthotopic glioma mouse model. Veledimex (AL) at doses of 225, 450 and 1200 mg/m²/day, once a day (QD) or twice a day (BID) was administered to 4 groups of female C57BL/6 mice. Depicted are mean (± SD) veledimex (AL) concentrations of 3 animals/time point/group in plasma (A) and brain (B). veledimex (AL) at 450 and 1200 mg/m²/day was administrated as BID doses daily to groups of 12 G261 orthotopic syngeneic female mice for 14 days. Terminal brain samples were collected on Days 3 and 14 prior to dosing. Depicted are mean (± SD) veledimex (AL) concentrations of 6 animals/time point/group in brain (C). At 24 h veledimex (AL) brain concentration was approximately 6- and 1.7-fold higher in GL261 mice than in normal C57BL/6 mice at 450 and 1200 mg/m²/day doses, respectively.

Veledimex Brain Penetration in Monkeys with Intact Blood Brain Barrier

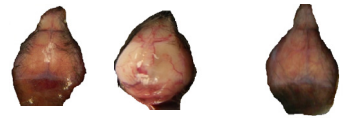
Parameters	Oral	IV
	Plasma	
Body Weight (kg)	4.1 ± 0.9	4.0 ± 0.9
Dose (mg/m²)	366 ± 82	120
C_{max} (ng/mL)	327 ± 142	7462 ± 1241
DN C_{max} (ng/mL)	0.9 ± 0.5	62.2 ± 10.3
T_{max} (h)	4 ± 1	0.08 ± 0.0
AUC_{0-t} (ng·h/mL)	5887 ± 2203	14145 ± 2983
DN AUC_{0-t} (ng·h/mL)	17.5 ± 8.8	117.9 ± 24.9
$AUC_{0-\infty}$ (ng·h/mL)	5916 ± 2210	14258 ± 3010
$t_{1/2}$ (h)	25.0 ± 6.1	32.2 ± 5.2
MRT_{0-t} (h)	18.4 ± 5.5	10.1 ± 1.6
CL (mL/h/kg)	NA	729 ± 157
V_d (mL/kg)	NA	33233 ± 5795
F(%)	14.1 ± 4.9	NA
	CSF	
C_{max} (ng/mL)	2.07 ± 0.91	15.8 ± 10.1
C_{max} ratio (CSF/plasma)	0.6 ± 0.2	0.21 ± 0.13
T_{max} (h)	4 (4, 24)	0.08 ± 0.0
AUC_{0-t} (ng·h/mL)	42.5 ± 17.4	73.7 ± 61.8
AUC_{0-t} ratio (CSF/plasma)	0.7 ± 0.2	0.49 ± 0.32

DN = dose normalized; NA = not applicable

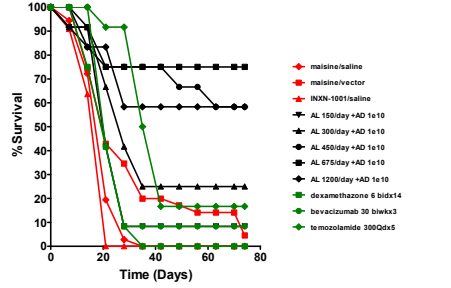
Summary of plasma and CSF PK parameters of veledimex in cynomolgus monkeys after a single oral dose of 120 mg veledimex in Maisine formulation on Day 1 and after an IV dose of 120 mg/m² on Day 8. Veledimex (AL) was administered as a single oral dose of 120 mg (six 20-mg capsules) to a group of 6 cynomolgus monkeys (3/sex). After a 7-day washout, veledimex was administered as a single intravenous (IV) bolus dose of 120 mg/m² to the same 6 monkeys. PK parameters are mean values (± SD) based on 6 monkeys (3/sex).

Results (Pharmacology)

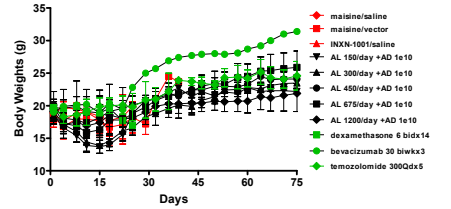
Effects of Ad-RTS-mIL-12 + Veledimex in the Orthotopic GL-261 Mouse



Ad-RTS-mIL-12 +Veledimex Results in Increased Survival over Current Standards of Care in the Mouse Orthotopic GL261 Glioma Model



Ad-RTS-IL-12 +Veledimex Does Not Adversely Affect Body Weight



Intra Tumor Regulated IL-12 Gene Delivery by Ad-RTS-mIL-12 Improves Survival in GL261 Glioma Model. 1 x 10^5 GL261 glioma cells were administered into the brain. Separate groups of 12 C57/B/6 mice each were randomly assigned to one of the treatment groups. On Day 1 mice were administered $1x10^5$ GL261 glioma cells i.t. On Day 5, Ad-RTS-mIL-12 at $1x10^{10}$ vp. + veledimex p.o. at 150-1200 mg/m²/day p.o. on a BID schedule was administered for 14 consecutive days and the time to disease progression and death was studied. Depicted in panel A is the survival results and panel B is the respective body weight. All values are expressed as the mean ± SEM.

Ad-RTS-mIL-12 + Veledimex Demonstrated Dose-related Increase in Survival in the Mouse GL261 Glioma Model

Treatment AL (mg/m²)	Number of Animals (N)	Mean Time-to-Endpoint (TTE) (Days)	Increase in Life Span (ILS) (%)[a]	Percent Survival Day ≥ 74
maisine/saline	36	17	----	0
maisine/vector	36	19	11.8	5
INXN-1001/saline	12	15	-11.8	0
AL 150/day bid +AD 1e10	24	17	0	8
AL 300/day bid +AD 1e10	12	26	52.9	25
AL 450/day bid +AD 1e10	12	>74[b]	>335[b]	58
AL 675/day bid +AD 1e10	12	>74[b]	>335[b]	75
AL 1200/day bid +AD 1e10	12	>74[b]	>335[b]	58
dexamethazone 3	12	13	-23.5	0
dexamethazone 6 bidx14	24	18	5.9	0
bevacizumab 15 biwkx3	12	18	5.9	0
bevacizumab 30 biwkx3	24	20	17.6	8
temozolamide 300 qdx5	12	35	105.9	17

[a] ILS=%T/C -100%, where T/C = quotient of mean TTE (days) of treated and control group, compared to Group 1; AL= veledimex p.o. BIDx14 day schedule; AD= Ad-RTS-mIL-12 1e10vp i.t.

Conclusions

- Veledimex exhibited dose-related increases in plasma and brain tissue exposure.
- Repeat veledimex dosing shows no accumulation in the brain (48 vs. 312 h).
- Veledimex brain concentration was ~1.7- and 6-fold higher in GL261 mice than in normal mice. No veledimex brain accumulation was observed.
- Dose-response was established, the optimal dose of orally administered veledimex was 450 mg/m² total dose p.o. + Ad-RTS-mIL-12 $1x10^{10}$ vp.
- Veledimex p.o. + Ad-RTS-mIL-12 $1x10^{10}$ vp improved survival over temozolomide, dexamethasone and bevacizumab.
- These findings support the utility of localized regulatable IL-12 production as an approach for the treatment of malignant glioma.

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