Phase I/II Study of Controlled IL-12 as Immunotherapy for Diffuse Intrinsic Pontine Glioma (DIPG)

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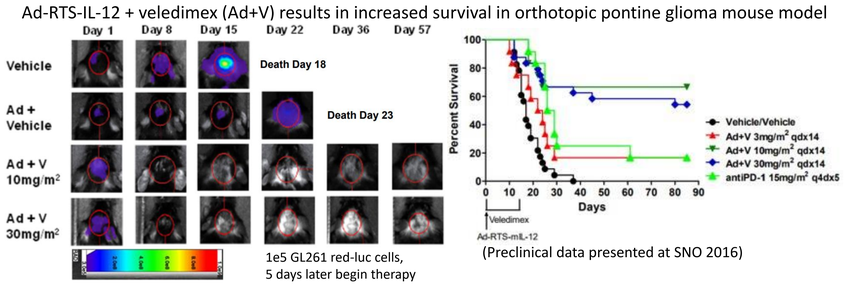
Introduction to DIPG

- Diffuse intrinsic pontine glioma (DIPG), the most frequent pediatric brainstem tumor, is one of the deadliest childhood cancers.
- Estimated incidence at ~1000 subjects annually worldwide with ~300-400 in the USA so it is considered an ultra-orphan disease
- Current standard of care treatment for DIPG is focal radiotherapy (RT), using a 1 cm margin to cover microscopic disease, to a total dose of 54–60 Gy administered over 6 weeks, usually in weekdays in 180–200 cGy fractions
  - Hyperfractionated RT provided no survival benefit over conventional RT
  - Radiation sensitizing agents have shown no benefit to date
- Recent important advances in its molecular characterization have not yet translated into improved treatments so new therapies are urgently needed
  - Mutation in histone H3 (H3.1K27M or H3.3K27M) (~80%) leading to epigenetic changes useful as biomarker, also alterations in *PDGFRA* (~36%) or *ACVR1* (~20%) genes
- Other therapies being tried include HDAC and ALK2 inhibitors, bevacizumab, CAR-T, etc.
- Edema is a common SAE and progressive disease affecting the respiratory center in the confined space of the pons is a frequent mode of death
- Thus DIPG remains an important unmet medical need in pediatric neuro-oncology

Background on Controlled IL-12

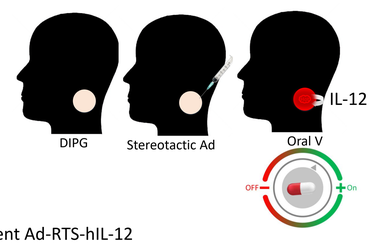
Ad-RTS-IL-12 + veledimex (Ad+V) results in increased survival in orthotopic pontine glioma mouse model



(Preclinical data presented at SNO 2016)

IL-12 controlled transcription under RheoSwitch Therapeutic System® (RTS®)

- Localized production of recombinant IL-12
- Proportional to oral dosing of veledimex
- Veledimex crosses the blood-brain barrier
- Real-time adjustment of IL-12
- Adapt to patient's needs
- Pause/stop in the event of cytokine release syndrome



Clinically, intratumoral injection of replication incompetent Ad-RTS-hIL-12 vector that is transcriptionally regulated by orally administered veledimex locally produces IL-12 which drives downstream production of endogenous IFNγ that in turn elicits a brisk cytotoxic immune response

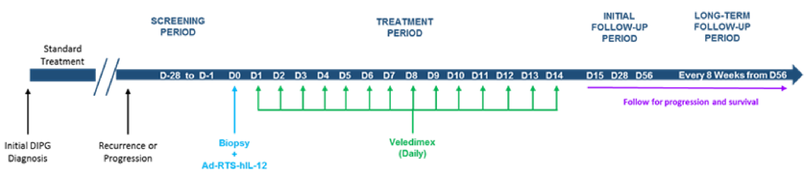
Clinical Study ATI001-103 arm 1 – supratentorial subjects (NCT03330197)

- Demonstrated safe stereotactic injection in 3/3 subjects
- Required before dosing of DIPG could begin

Study Design

Clinical Study ATI001-103 Amendment 2 – DIPG (NCT03330197)

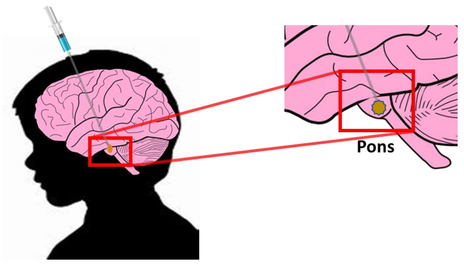
Multicenter, open-label study, of Ad (2 x 10^{11}viral particles IT) + V doses (10 mg and 20 mg PO, BSA adjusted), focusing on DIPG (arm 2)



- Key eligibility criteria (arm 2):
  - M/F, ≤ 21 years-of-age
  - Clinical presentation of DIPG involving ~2/3 of pons on MRI
  - ≥ 2 and ≤ 10 weeks post standard focal radiotherapy (5400-5960 cGy)
  - Maximum dexamethasone of 1 mg/m²/day), on a stable or decreasing dose of steroids for the last 7 days
  - Lansky score ≥ 50, Karnofsky performance status > 50 or ECOG score ≤ 2
  - Adequate bone marrow, liver and kidney function
- Standard 3+3 dose escalation design
- Primary Objective: Safety and tolerability
- Secondary objectives: RP2D, PK of V, cellular/humoral immune responses, assess response (ORR, PFS), OS
- Exploratory objectives: Biomarkers include viral shedding, H3.3 mutation status, serum cytokine levels (IL-12, IFNγ), and neutralizing antibody response
- Safety Review Committee (SRC) may de-escalate by 5 mg if ≥2 DLTs (dose limiting toxicities) are observed or discontinue investigation. After phase 1 dose escalation, SRC may expand cohort by up to 30 subjects for phase 2 component of study
- Clinical study recently received Rare Pediatric Disease Designation from FDA
- Enrollment is ongoing

First DIPG Subject Dosed

Successful injection into the pons
- **No significant edema or mass effects post procedure**
- **No DLTs, SAEs or SUSARs during active study period**



Subject 004: 5-year-old male diagnosed November 2019 – biopsy showed a H3.3K27M-mutant diffuse midline glioma, WHO Grade IV consistent with DIPG with pre-existing midline cyst, status post 1 line of therapy (photon radiotherapy), and assessed as unifocal disease at study entry

First Subject was dosed on 07 April 2020 (Day 0):

- Stereotactic injection was performed administering 0.1 mL Ad-RTS-hIL-12 (2 x 10e11 viral particles) with a modified technique to enable a contralateral approach to avoid the cyst
- After completion of the procedure, subject was able to move all extremities after waking

Days 0-4:

- Alert, responsive to questions, reports of mild right sided facial muscle weakness (esotropia), subject had a constellation of findings (mild-moderate fever, nausea, leukopenia, lymphopenia, borderline hypotension and neutropenia) consistent with low-grade CRS

Day 14:

- No SAEs and no dexamethasone during active dosing (Days 0-14)
- Was clinically stable and did well overall. Mild right xerostomia, continued right facial partial paresis, and notably partial eyebrow loss. Speculatively, the latter finding is suggestive of mild alopecia areata, an immune related effect (mild cross reactivity) Subject received 14/14 doses (93% compliance) of veledimex 10 mg

Day 42:

- Prior cranial nerve 12 neuropathy improved, partial right lid closure was unchanged, left esotropia (cranial nerve 6) slightly improved, and ambulation improved – was running and jumping in family apartment. No further alopecia noted. Lack of appetite persisted but gained back 1 lb from nadir

Day 56:

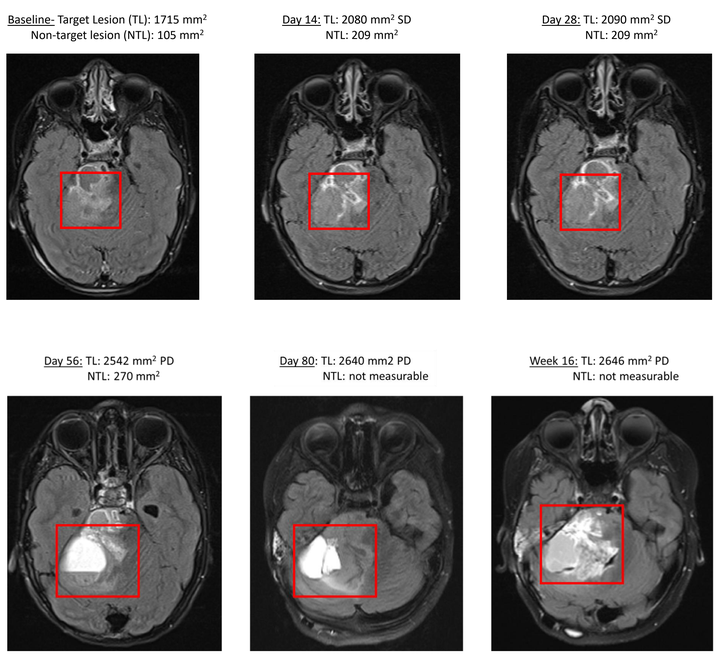
- Clinical progression noted
- Neurosurgery consult for management of increasing cyst size. VP shunt inserted

Week 17:

- Subject died from Progression of Disease (PD)

Representative Serial MRI Scans

- Baseline: Expansile infiltrative T2 hyperintense lesion of pons, predominantly right-sided, extending caudally into upper medulla as well as through the right cerebellar peduncle, with compression of fourth ventricle and postoperative changes; 1 target and 1 non-target lesion identified
- Day 14: MRI showed some relative increase in pre-existing cyst size with no appreciable change in the solid component of the tumor (SD)
- Day 28: Minimal increase in TL (SD) and no change in NTL
- Day 56: Further increase in size of cystic component with peripheral mass effect with lesser increase in the solid, nodular component of the tumor on MRI (PD). High signal in cyst seen in T2 FLAIR sequence in conjunction with T1 findings (not shown) is presumably due to increased proteinaceous content (e.g., hemorrhage and/or sloughing)
- Day 80: Increase in TL (PD) with decrease in size of cyst status post shunt insertion
- Week 16: Mild increase in TL (PD)



Axial post contrast T2 FLAIR fast spin echo sequences. Measurements presented as sum of biperpendicular product diameters (SPD)

Safety Results

The cumulative TEAEs for Study 103 are summarized in the table below:

Preferred Terms	ATI001-103 Safety Results			
	Arm 1 (N=3)		Arm 2, (DIPG, N=1)	
	Any Grade	>= Grade 3	Any Grade	>= Grade 3
TEAEs Related to Ad+V				
Any related AE	3 (100)	1 (33.3)	1 (100)	0
Anemia	1 (33.3)	0	0	0
Anxiety	1 (33.3)	0	0	0
Aphasia	1 (33.3)	0	0	0
Confusional state	1 (33.3)	0	0	0
Decreased appetite	1 (33.3)	0	0	0
Dehydration	1 (33.3)	0	0	0
Dizziness	1 (33.3)	0	0	0
Fatigue	1 (33.3)	0	0	0
Headache	2 (66.7)	0	0	0
Hyponatraemia	2 (66.7)	0	0	0
Lymphocyte count decreased	2 (66.7)	1 (33.3)	1 (100)	0
Nausea	1 (33.3)	0	1 (100)	0
Neutrophil decreased	0	0	1 (100)	0
Optic nerve disorder	1 (33.3)	0	0	0
Platelet count decreased	2 (66.7)	0	0	0
Pyrexia	1 (33.3)	0	0	0
Tachycardia	0	0	1 (100)	0
Vomiting	2 (66.7)	0	1 (100)	0
WBC count decreased	2 (66.7)	0	1 (100)	0
Cytokine Release Syndrome[1]	2 (66.7)	0	1 (100)	0

[1]Ziopharm Cytokine Release Syndrome Working Definition

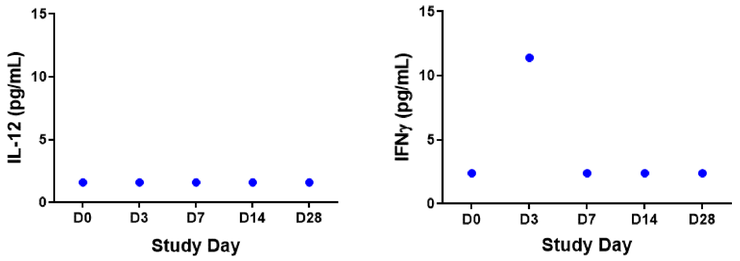
- Safety results for the subject with DIPG (arm 2) were generally similar to the 3 pediatric subjects with supratentorial tumors (arm 1)
  - No DLTs were observed
  - No SAEs or SUSARS were reported
  - Most of the related AEs were expected; all were mild to moderate in severity and most were promptly reversible upon withholding of veledimex doses
  - Overall, monotherapy was well-tolerated at current dose levels

Biomarker Results

Viral shedding

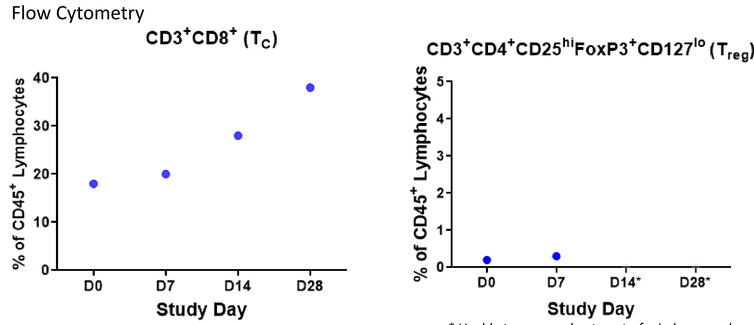
- Evaluated using a qPCR assay for RTS® vector DNA
- Buccal swab, saliva, plasma, serum, urine and feces specimens were studied at Day 0 and Day 3
- All specimens collected showed either Not Detected or <LLOQ (25 copies per reaction)

Serum cytokine levels



(The peak in endogenous downstream IFNγ at Day 3 suggests a perhaps earlier rise in serum recombinant IL-12 might not have been detected by the sampling schedule used for older pediatric supratentorial brain tumor subjects from Arm 1)

Flow Cytometry



* Unable to process due to out-of-window sample age for this antibody panel (pandemic effect)

Discussion and Conclusions

Initial safety findings for the first subject dosed in arm 2 (DIPG) were encouraging:

- Ad-RTS-hIL-12 vector was safely administered into the pons by stereotactic injection
  - No significant edema or mass effects in the pons on post procedure MRI
- Therapy was clinically well-tolerated at the initial dose level (V 10 mg/day)
  - High veledimex compliance. No DLTs, SAEs or SUSARs occurred during active study period
  - AEs were similar to adult and older pediatric supratentorial brain tumor subjects in being mild to moderate and predominantly reversible upon withholding of veledimex doses
- Immune activation was supported by the following:
  - Although an increase in serum recombinant IL-12 was not detected at the timepoints sampled, endogenous downstream IFNγ level peaked at 11.4 pg/mL on Day 3. Sampling on Days 1-2 may be indicated in future studies to detect an increase in serum recombinant IL-12 in certain subjects
  - Flow cytometry in peripheral blood showed an increase in cytotoxic T lymphocytes (T_C) between Days 7 and 28. No change in regulatory T lymphocytes (T_{reg}) was detected
  - Speculatively, partial eyebrow loss suggestive of immune-mediated alopecia areata
- Survival of the first subject dosed was within the historical reference range as expected for the initial dosing cohort
- Enrollment is anticipated to increase as pandemic restrictions at the clinical sites ease
- Dose-escalation of V from 10 to 20 mg/day is ongoing as planned per protocol