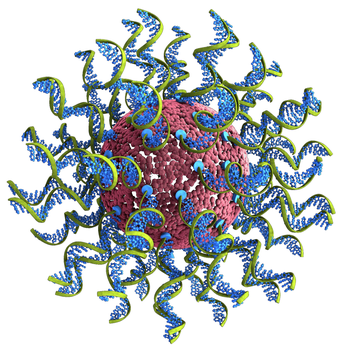
AST-008, a TLR9 Agonist Spherical Nucleic Acid, Activated NK Cells, T Cells and Cytokines in Healthy Subjects in a Phase 1 Clinical Trial

Weston L Daniel,[1] Ulrike Lorch,[2] Simon Coates,[2] Alice S. Bexon,[3] and Scott Mix[1]
[1]Exicure Inc., 8045 Lamon Ave, Skokie, Illinois. [2]Richmond Pharmacology Limited, London, United Kingdom. [3]Bexon Clinical Consulting, Montclair, New Jersey.
Corresponding author: Weston L Daniel (wes@exicuretx.com)

BACKGROUND

AST-008



- AST-008 is a toll-like receptor 9 (TLR9) agonist oligonucleotide in a proprietary spherical nucleic acid (SNA) format with immune-stimulatory properties
- SNAs are dense, radial arrangements of nucleic acids that have useful properties as compared to linear oligonucleotides (i.e., oligonucleotides not in the SNA format), notably increased cellular uptake and an optimal presentation of the oligonucleotides for TLR9 agonism
- AST-008 is designed to enter into and activate immune cells to elicit an immune response to treat solid tumors in combination with a checkpoint inhibitor
- AST-008 has potent antitumor activity as a monotherapy and synergizes with anti-PD-1 antibody therapy in several preclinical tumor models

OBJECTIVES

- **Primary**
  - To evaluate the safety and tolerability of AST-008 after single subcutaneous (SC) doses
- **Secondary**
  - To recommend a dose and regimen for further development
  - To determine the pharmacokinetics (PK) of AST-008 in plasma and urine
  - To determine the pharmacodynamics (PD) of AST-008 after SC doses
  - To determine the effect of AST-008 on QTc interval

METHODS

- AST-008 was evaluated in a Phase 1 study under protocol AST-008-101
- Four dose levels of AST-008 were evaluated in four cohorts. Each cohort included four volunteers, and all received a single dose of AST-008. The dose levels were 5, 10, 12.5 and 18.8 µg/kg
- Peripheral blood cytokine concentrations and cell activation were measured with a Randox Evidence Investigator or ELISA, and a Beckman Coulter Navios flow cytometer, respectively
- Plasma concentrations of AST-008 were assessed with a solid phase hybridization assay with electrochemiluminescent detection; urine concentrations were assessed with a liquid chromatography method and fluorescence based detection

POPULATION

- Healthy volunteers age 18 to 40 with body mass index of 18 to 25 kg/m^2
- Subjects with significant medical history or significant abnormalities, a recent history of tobacco, drugs of abuse, prescription medications including corticosteroids or other immunosuppressive drugs, or other investigational products were excluded

ADVERSE EVENTS

AEs/ARs Observed in the Study by Dose - All Were Grade 1

System Organ Class	Preferred Term (MedDRA 21.0)	5 µg/kg (N=4) n (%)	10 µg/kg (N=4) n (%)	12.5 µg/kg (N=4) n (%)	18.8 µg/kg (N=4) n (%)	Overall (N=16) n (%)
Blood and lymphatic system disorders	Lymphadenopathy	-	1 (25.0)	-	-	1 (6.3)
Eye disorders	Eye pain	1 (25.0)	-	-	-	1 (6.3)
General disorders and administration site conditions	Influenza like illness	-	3 (75.0)	-	1 (25.0)	4 (25.0)
	Injection site reactions (AR)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	16 (100.0)
	Pyrexia	-	-	2 (50.0)	4 (100.0)	6 (37.5)
Metabolism and nutrition disorders	Decreased appetite	-	-	-	1 (25.0)	1 (6.3)
Musculoskeletal and connective tissue disorders	Back pain	-	-	1 (25.0)	-	1 (6.3)
	Muscle twitching	1 (25.0)	-	-	-	1 (6.3)
	Myalgia	-	-	-	2 (50.0)	2 (12.5)
Nervous system disorders	Dizziness	-	-	1 (25.0)	1 (25.0)	2 (12.5)
	Headache	1 (25.0)		1 (25.0)	2 (50.0)	4 (25.0)
	Hyperaesthesia	-	-	-	1 (25.0)	1 (6.3)
Respiratory, thoracic and mediastinal disorders	Cough	-	-	-	1 (25.0)	1 (6.3)
Vascular disorders	Flushing	-	-	1 (25.0)	-	1 (6.3)

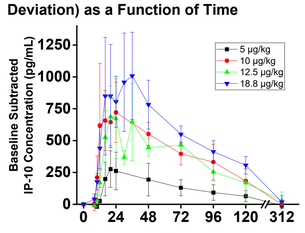
- No serious adverse events or dose limiting toxicity were observed
- Non-adverse CRP increases, neutropenia, lymphopenia and lymph node enlargement (other than the instance noted above) occurred

CYTOKINE EXPRESSION AND LYMPHOCYTE ACTIVATION

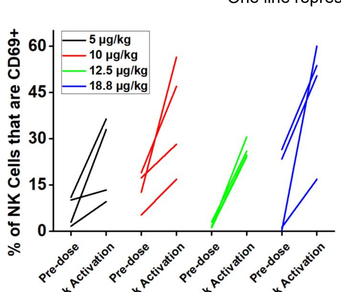
Peak Cytokine Response (Mean ± Standard Deviation, Expressed in pg/mL) as a Function of Dose (Baseline Subtracted)

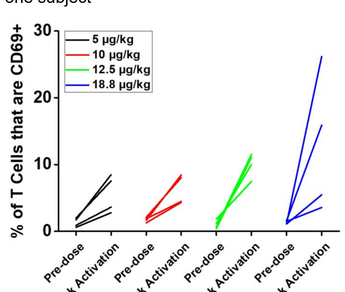
Cytokine	5 µg/kg	10 µg/kg	12.5 µg/kg	18.8 µg/kg
IL-12 (p40)	109 ± 71	205 ± 140	288 ± 60	289 ± 166
IL-1RA	895 ± 242	2083 ± 609	1165 ± 479	1764 ± 558
IP-10	333 ± 132	936 ± 93	815 ± 185	1190 ± 285
IL-6	32 ± 8	139 ± 62	80 ± 72	93 ± 82
MCP-1	333 ± 125	906 ± 457	348 ± 49	489 ± 194
TNFα	4 ± 3	18 ± 27	3 ± 3	48 ± 88
IFNα	18 ± 24	8 ± 4	7 ± 9	4 ± 6
IFNγ	3 ± 1	16 ± 10	4 ± 3	5 ± 2

IP-10 Expression (Cohort Mean ± Standard Deviation) as a Function of Time



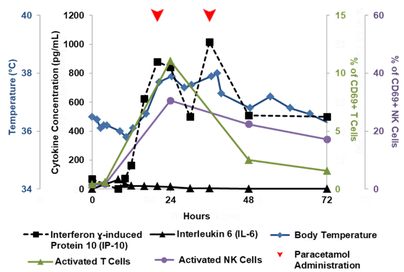
AST-008 Activated Natural Killer (NK) and T Cells at All Dose Levels Tested
One line represents one subject





A SUBJECT "STORY"

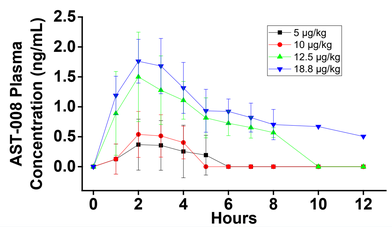
Subject Receiving 12.5 µg/kg Dose Exhibits Temporally Reasonable Relationships Between Cytokines, Body Temperature and Cell Activation



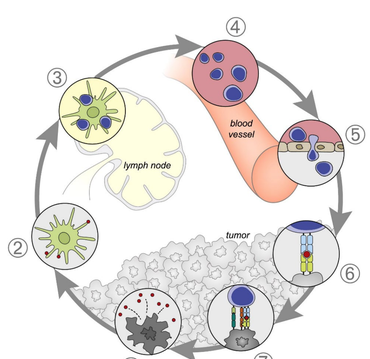
AST-008 PK

AST-008 Exposure Increases in an Approximately Dose Proportional Manner

Dose (µg/kg)	5	10	12.5	18.8
$AUC_{0\text{-}24hr}$ (ng × hr/mL)	2.302	2.084	4.762	8.170
C_{max} (ng/mL)	0.74009	0.73726	1.3336	1.8234
T_{max} (hr)	2.52	2.00	2.00	2.04



AST-008 / PEMBROLIZUMAB IN THE CANCER IMMUNITY CYCLE



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Step 1: Release of cancer cell antigens
Facilitated by activated NK cells ✔

Step 2: Cancer antigen presentation
Facilitated by TNF ✔

Step 3: Priming and activation
Facilitated by IL-12 ✔ Inhibited by PD-1 / PD-L1 ✔

Step 4: Trafficking to T cells to tumors
Facilitated by IP-10 (CXCL10) ✔

Step 6: Recognition of cancer cells by T cells
Anticipated result in cancer patients from the effects observed in steps 1-4

Step 7: Killing of cancer cells
Facilitated by IFNγ ✔ Inhibited by PD-1 / PD-L1 ✔

✔ = Observed in AST-008 Phase 1 trial
✔ = Prevented by pembrolizumab

CONCLUSIONS

- AST-008 was well tolerated and elicited no serious adverse events or dose limiting toxicity at the doses tested
- AST-008 is a potent innate immune activator and exhibits pharmacodynamic properties that are expected to result in anti-tumor effects in patients with cancer
- AST-008 plasma exposure was roughly dose proportional; peak AST-008 plasma concentrations were observed before peak pharmacodynamic effects. AST-008 was not detected in urine
- Phase 1b/2 study of intratumorally-dosed AST-008 in combination with pembrolizumab in cancer patients is ongoing. Non-melanoma skin cancers are of particular interest in this study