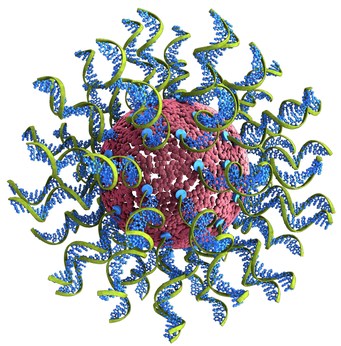
A Phase 1 Study of the Safety, Pharmacokinetics and Pharmacodynamics of AST-008, a TLR9 Agonist Spherical Nucleic Acid, in Healthy Subjects

Weston L Daniel,[1] Ulrike Lorch,[2] Simon Coates,[2] and Scott Mix[1]
[1]Exicure Inc, 8045 Lamon Ave, Skokie, Illinois, United States. [2]Richmond Pharmacology Limited, London, United Kingdom
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 tumours in combination with a checkpoint inhibitor
- AST-008 has potent antitumour activity as a monotherapy and synergizes with anti-PD-1 antibody therapy in several preclinical tumour 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 1a 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 and urine concentrations of AST-008 were assessed with a peptide nucleic acid probe/liquid chromatography assay

POPULATION

- Healthy volunteers age 18 to 40 with body mass index of 18 to 25 kg/m^2
- Subjects with 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, at Least Possibly Related to AST-008, Observed in the Study

System Organ Class	Preferred Term (MedDRA 20.1)	Grade by CTCAE V4.03 n (%) Grade 1	Grade 2	Grade 3	Grade 4	All Grades
Blood and lymphatic system disorders	Lymphadenopathy	12 (75)	-	-	-	12 (75)
	Lymphopenia	6 (38)	5 (32)	2 (13)	-	13 (81)
	Neutropenia	3 (19)	6 (38)	-	1 (6)	10 (63)
General disorders and administration site conditions	Hot flush	2 (13)	-	-	-	2 (13)
	Influenza like illness	2 (13)	-	-	-	2 (13)
	Injection site erythema	13 (81)	-	-	-	13 (81)
	Injection site pain	11 (69)	-	-	-	11 (69)
	Injection site swelling	9 (56)	-	-	-	9 (56)
	Pyrexia	7 (44)	2 (13)	-	-	9 (56)
Investigations	CRP increase	4 (25)	-	-	-	4 (25)
Musculoskeletal and connective tissue disorders	Myalgia	3 (19)	-	-	-	3 (19)
Nervous system disorders	Dizziness	2 (13)	-	-	-	2 (13)
	Headache	6 (38)	-	-	-	6 (38)

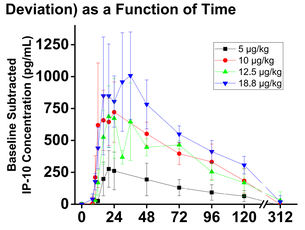
- One instance each of Grade 1 back pain, chills, cough, decreased appetite, eye pain, hyperaesthesia, muscle twitching, nausea, photophobia, and sinus tachycardia was observed
- No serious adverse events or dose limiting toxicity were observed

CYTOKINE EXPRESSION AND LYMPHOCYTE ACTIVATION

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

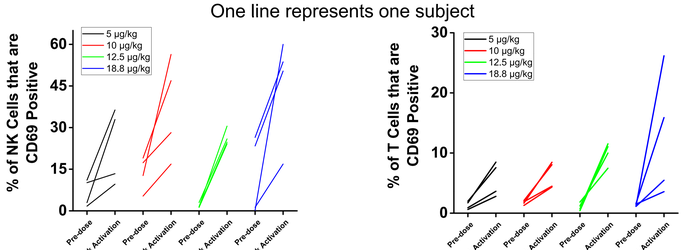
Cytokine	5 µg/kg	10 µg/kg	12.5 µg/kg	18.8 µg/kg
IL-12 (p40)	109 ± 71	205 ± 140	288 ± 60	289 ± 166
IL-1RA	895 ± 242	2083 ± 609	1165 ± 479	1764 ± 558
IP-10	333 ± 132	936 ± 93	815 ± 185	1190 ± 285
IL-6	32 ± 8	139 ± 62	80 ± 71	93 ± 82
MCP-1	333 ± 125	906 ± 457	348 ± 49	489 ± 194
TNFα	4 ± 3	18 ± 27	3 ± 3	48 ± 88
IFNα	18 ± 24	8 ± 4	5 ± 6	4 ± 6
IFNγ	3 ± 1	16 ± 10	2 ± 0	5 ± 2

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



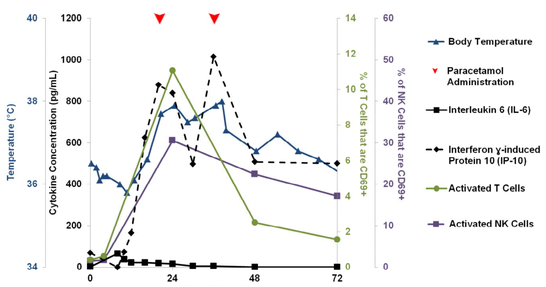
AST-008 Activated Natural Killer (NK) and T Cells at All Dose Levels Tested

One line represents one subject

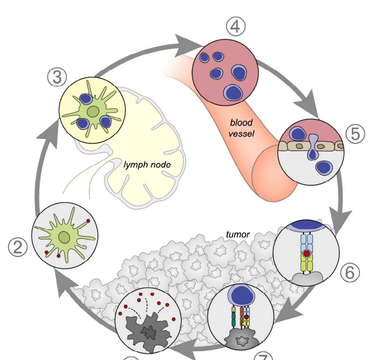


A SUBJECT "STORY"

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



AST-008 / PEMBROLIZUMAB IN THE CANCER IMMUNITY CYCLE



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 tumours
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 ✔

Immunity, Volume 39, Issue 1, 25Jul2013, Pages 1-10

✔ = 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-tumour effects in patients with cancer
- AST-008 was not detected in the plasma or urine of any subject
- Preparation of a Phase 1b/2 study of AST-008 in combination with pembrolizumab in cancer patients is ongoing