

Biomarker identification in serum samples from patients with recurrent cervical cancer treated with ADXS11-001 immunotherapy

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Abstract

Cervical Cancer is the third most common cancer worldwide and is almost always caused by human papilloma virus (HPV) infection. The HPV genes E6 and E7 are known to be oncogenes that promote cellular changes in infected cells leading to malignant transformation. We have previously reported that ADXS11-001 immunotherapy, a live attenuated *Listeria monocytogenes* (Lm), bioengineered to secrete a LLO-E7 fusion protein, can be safely administered to patients with late stage cervical cancer. In a Phase 2 study being conducted in India, 110 patients with recurrent cervical cancer received 264 doses of ADXS11-001. In this study, serum samples were collected from each patient at three different time points: pre-dose; 2h and 4h post-dose of ADXS11-001 immunotherapy. The initial analysis of 18 serum samples using 46-biomarker multi-analyte human inflammation MAPv1.0 (Myriad RBM) showed differential increases in cytokine and chemokine levels in each sample at 2 and 4 hours post-dosing with ADXS11-001. More than a 15-fold increase was observed in the level of cytokines (IL-6, IL-8, IL-10 and TNF- α) and chemokines (MIP-1 α , MIP-1 β and MCP-1), indicating strong stimulation of innate immunity. We will report our evaluation of RNA from serum samples collected from patients at pre- and post-dosing of ADXS11-001 using a genomics-based approach and evaluate correlation with changes in cytokine and chemokine levels. We will further report the association of changes in the expression of cytokines or other serum factors with the severity of adverse events as well as clinical responses. In conclusion, RNA or protein expression profiles from patient serum may help in identifying biomarkers which may be employed as a screening tool in future studies to predict efficacy, monitor side effects, and suggest more precise toxicity management for patients treated with ADXS11-001 immunotherapy.

Discovery And Validation Overview

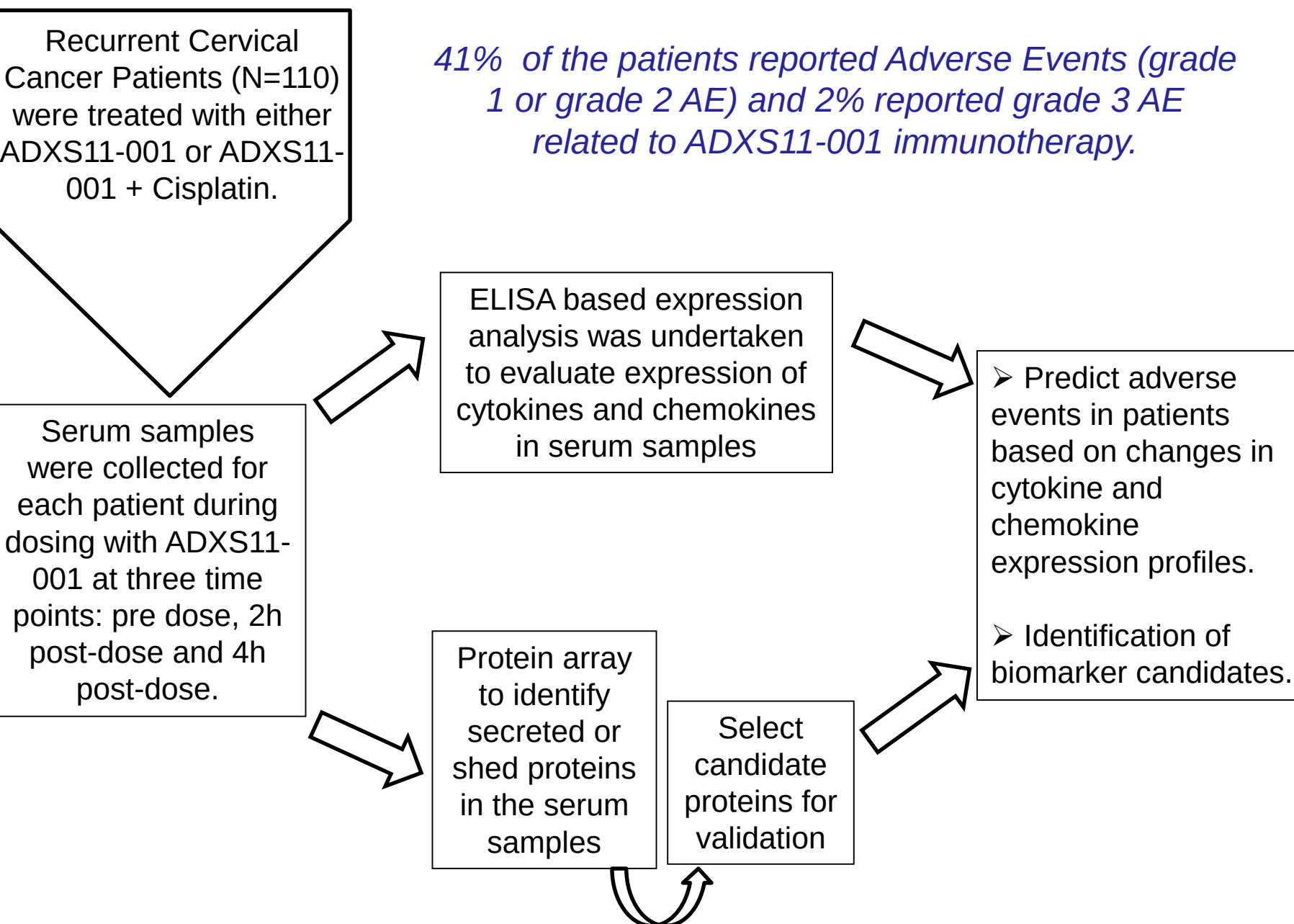


Figure 1. Workflow showing the discovery and validation strategy being used to identify biomarkers in serum samples from patients treated with ADXS11-001 immunotherapy.

Relative Increase In Cytokines And Chemokines Post-Dose

Cytokine/Chemokines	Relative Fold Increase (2h)	Relative Fold Increase (4h)
IL-6	34.00	32.68
IL-8	22.50	12.80
IL-10	28.47	33.78
IL-18	0.57	0.63
MIP-1 α	3.00	2.10
INF- γ	2.10	1.80
MCP-1	35.52	23.94
TNF- α	19.21	11.78
TNF-R2	1.94	1.84

Figure 2. Serum samples analyzed thus far using ELISA shows relative increase in cytokines and chemokines at 2 and 4 hours post dosing. More than a 15-fold increase is observed in the level of cytokines (IL-6, IL-8, IL-10 and TNF- α) and chemokine (MCP-1) post-administration of ADXS11-001, indicating strong stimulation of innate immunity.

Cytokine And Chemokine Profile For ADXS11-001 Treated Patient Serum Samples

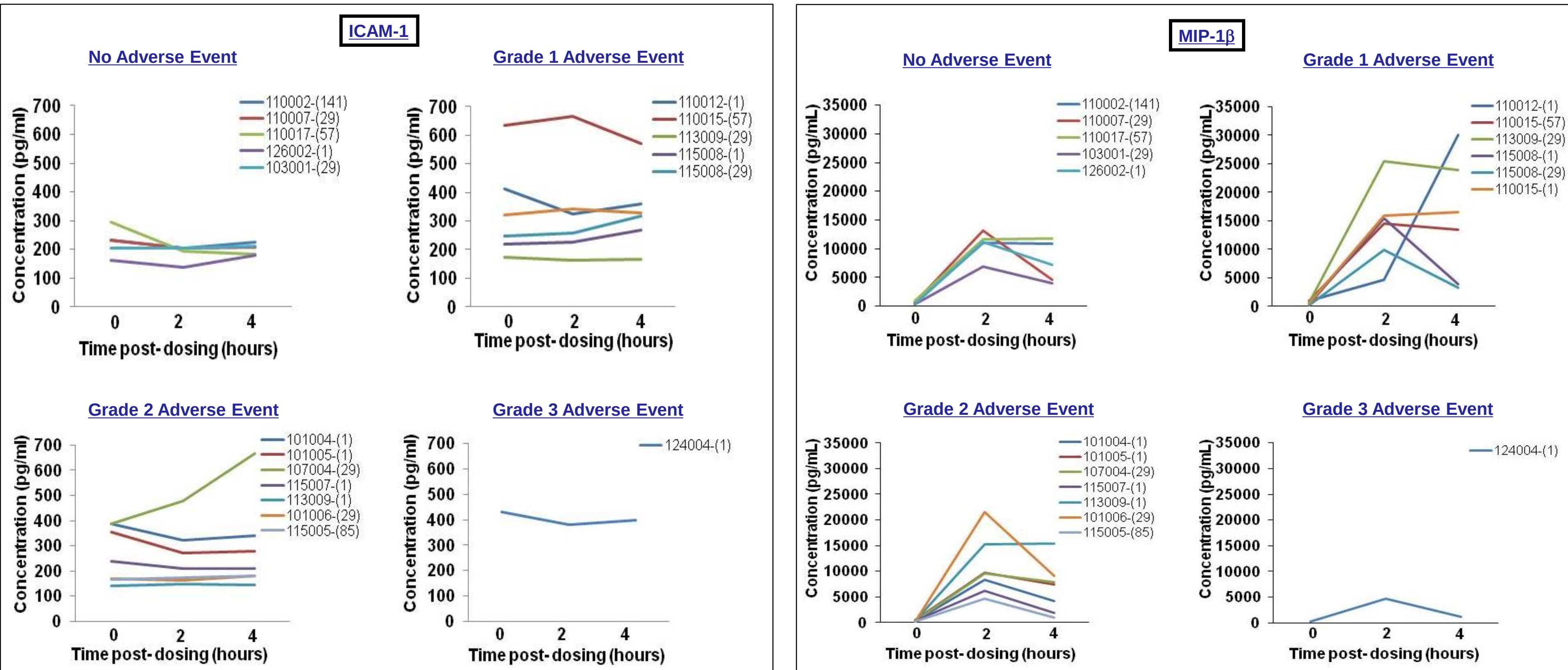


Figure 3. Line plots showing changes in expression of Intracellular Adhesion Molecule-1 (ICAM-1) and Macrophage Inflammatory Protein (MIP-1 β) in a subset of clinical samples analyzed thus far in patients treated with ADXS11-001 immunotherapy. Serum samples from a patient with grade 3 AE and from about 50% of patients with grade 1 AE or grade 2 AE showed ICAM-1 levels above normal range (78-339 pg/ml). Serum samples from patients with no AE showed ICAM-1 levels within the normal range. Also, an 18 fold increase in MIP-1 β expression is observed post ADXS11-001 dosing.

ICAM1/MIP-1 β Ratio May Be Used To Predict Adverse Events In Patients When Treated With ADXS11-001

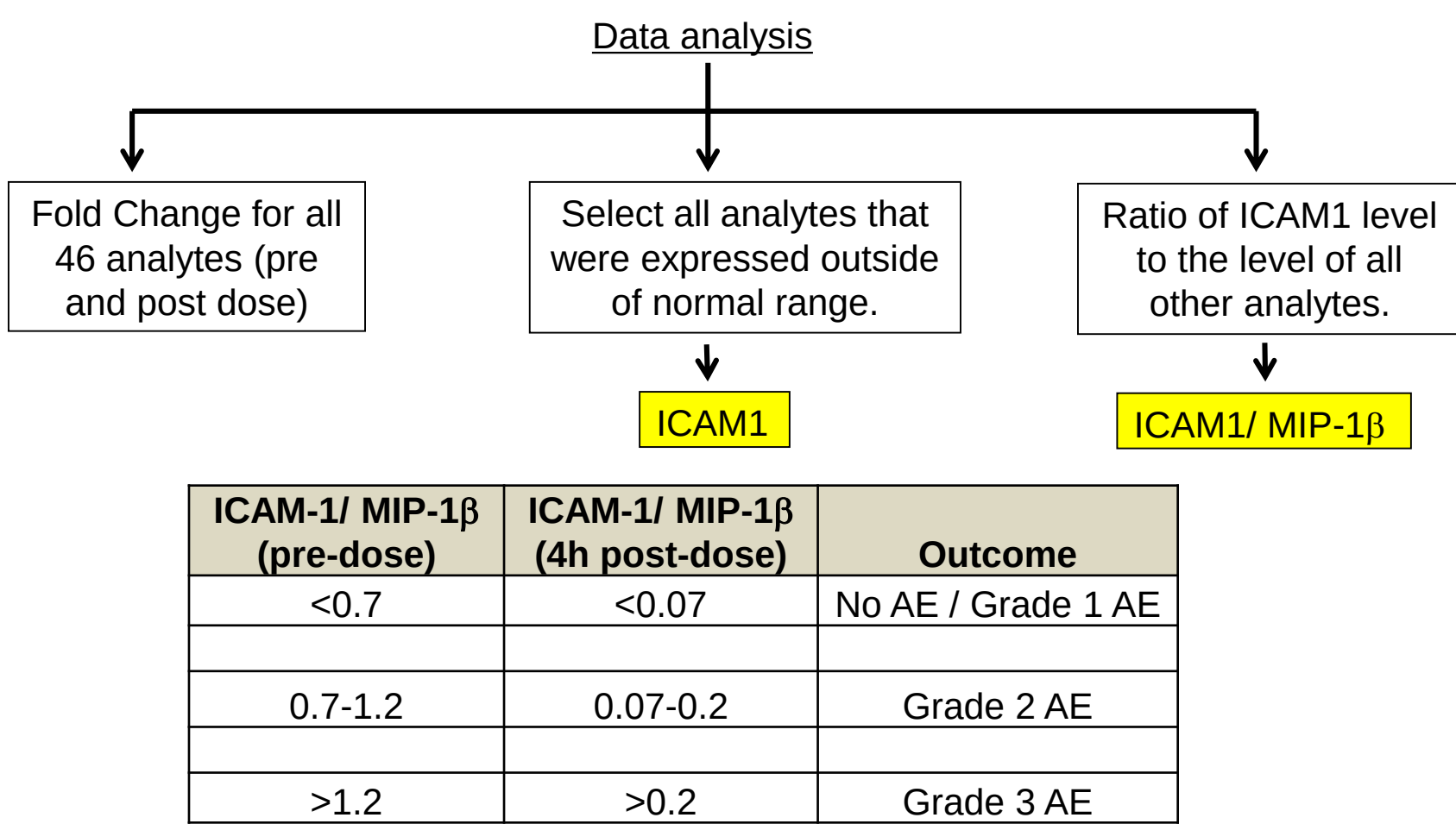


Figure 4. Flow chart showing steps used in the data analysis process. Using the pre-dose ratio of ICAM-1/MIP-1 β , we can predict adverse events (no AE, grade 1 AE, grade 2 AE or grade 3 AE) in about 84% of patients and by using 4h post-ADXS11-001 immunotherapy ratio of ICAM-1/MIP-1 β expression we can predict adverse events in about 77% patients.

Statistical Analysis

(Pre-dose)	Adverse Event (AE)	ICAM1/MIP-1 β (pre-dose)	ICAM1/MIP-1 β (4h post-dose)	(4h post-dose)
P=0.003	Grade 3 AE	1.98	0.347	P=0.03
	No AE	0.57	0.052	
	No AE	0.55	0.021	
	No AE	0.40	0.045	
	No AE	0.32	0.016	
	No AE	0.41		
	Grade 1 AE	0.39	0.012	
	Grade 1 AE	0.68	0.020	
	Grade 1 AE	0.67	0.042	
	Grade 1 AE	0.24	0.007	
P=0.01745	Grade 1 AE	0.66	0.069	P=0.04034
	Grade 1 AE	0.94	0.098	
	Grade 2 AE	1.02	0.080	
	Grade 2 AE	0.74	0.038	
	Grade 2 AE	1.18	0.084	
	Grade 2 AE	0.77	0.108	
P=0.473	Grade 2 AE	0.38	0.009	P=0.03
	Grade 2 AE	0.79	0.020	
	Grade 2 AE	0.56	0.187	

Figure 5. Unpaired Student's T test was used for statistical analysis. Significant difference was observed in the ratio of ICAM-1/ MIP-1 β for grade 1 AE or no AE compared to grade 2 AE.

Conclusions

- Increase in expression of cytokines (IL6, IL-8, IL10, INF- γ and TNF- α) and chemokines (MIP-1 α , MIP-1 β and MCP-1) indicates activation of innate immunity by ADXS11-001 immunotherapy.
- The magnitude of adverse events in patients may be related to the ratio of ICAM-1/ MIP-1 β expression in serum samples.

Future Direction

- Cytokine profiling of serum samples from other patients in the study who experienced side effects related/ possibly-related to ADXS11-001 immunotherapy.
- Data analysis to predict treatment response based on cytokine profile.
- Data analysis to differentiate between patients treated with ADXS11-001 alone or AdXS11-001 + Cisplatin.
- Protein array analysis to identify novel biomarkers in serum samples collected from patients treated with ADXS11-001.