Poster #366

TD-8236, a lung-selective inhaled pan-JAK inhibitor, inhibits gene expression related to severe asthma and exhaled nitric oxide (FeNO) in 3-D airway epithelium liquid interface (ALI) cultures derived from asthmatic donors

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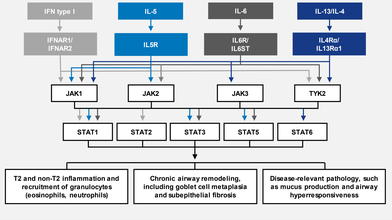
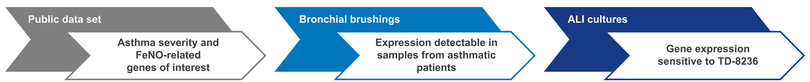
INTRODUCTION



- Asthma is a heterogeneous inflammatory lung syndrome with multiple endotypes (e.g., T2-high and T2-low) and disease severity associated with many Janus kinase (JAK)-dependent cytokines (e.g., IL-13, IL-4, IL-5, TSLP, IFNγ, IL-6, and IL-23)[1]
- Gene expression studies of bronchial brushings from asthmatic patients have contributed to the understanding of heterogeneous molecular drivers underlying disease[2]
- Fractional exhaled nitric oxide (FeNO), a widely used biomarker of T2-high inflammation that reflects both target engagement and disease activity in early clinical development, can be driven by NOS2 gene expression in airway epithelial cells[2]
- Biomarkers of T2-low lung inflammation in bronchial brushings have not been widely characterized, but the non-T2 cytokine pathways associated with disease[3] are sensitive to JAK inhibition
- TD-8236 is a novel, lung-selective, pan-JAK inhibitor designed for inhalation with minimal systemic exposure[4] that has potent in vitro activity against relevant cytokines implicated in T2-high and T2-low endotypes of moderate to severe asthma[5]

AIM

- Identify genes of interest from public data sets related to asthma severity and FeNO level
- Demonstrate detection of genes of interest in bronchial brushings from asthmatic donors with a range of FeNO levels
- Demonstrate inhibition of expression of genes of interest in TD-8236-treated airway epithelium air-liquid interface (ALI) cultures from asthmatic donors



METHODS

Microarray data analyses

- Microarray (GSE63142)[2] data, which included 27 healthy controls, 72 nonsevere asthmatics, and 56 severe asthmatics, was downloaded from Gene Expression Omnibus
- Samples were grouped based on either asthma severity (healthy control, nonsevere, and severe) or FeNO level (healthy control, <30 ppb FeNO [low], and >30 ppb FeNO [high]), or correlated to donor FeNO values (ppb)
- Samples were quality checked using arrayQualityMetrics (R QC package), and QC-passed samples were processed through limma (R package)
- Samples were background corrected and normalized using quantile normalization, and intensities of the replicate genes or probes were averaged for differential expression analysis
- Using the Empirical Bayes Statistics for Differential Expression method, fold change and p-values were calculated, and the Bonferroni and Hochberg correction method was used to generate adjusted p-values for each severity or FeNO level comparison set
- Spearman correlations were performed for normalized intensities of genes from each sample vs FeNO values at a threshold false discovery rate of 5%

Bronchial epithelial brushings from asthmatic donors

- RNA from bronchial epithelial brushings of 13 steroid-naïve, mild asthmatic donors with a mean FeNO score of 56.8 ppb was obtained from the Medicines Evaluation Unit (MEU; Manchester, England)
- RNA was analyzed for gene expression using the nCounter® Immunology Panel (Human V2) and the Vantage 3D RNA Cellular Signaling Panel (NanoString Technologies, Seattle, WA)
- Real-time PCR was performed using TaqMan gene expression assays (Thermo Fisher, Waltham, MA) on StepOnePlus (Applied Biosystems, Foster City, CA)
  - Relative quantification of gene expression was normalized to GAPDH and calculated using the $2^{-\Delta\Delta C_t}$ method

ALI culture assays

- Asthmatic donor tracheal/bronchial epithelial cells were grown and differentiated on a porous membrane support, allowing an air-liquid interface with warmed culture medium below the cells and a gaseous test atmosphere above (EpiAirway™ system, MatTek, Ashland, MA)
- TD-8236 was diluted in dimethyl sulfoxide (DMSO) to a final DMSO concentration of 0.1%; 0.1% DMSO alone served as vehicle control
- Cultures were exposed to prewarmed media containing no treatment (basal) or 1, 10, or 100 ng/ml IL-13 (R&D Systems, Inc., Minneapolis, MN) for up to 8 days

NanoString data analyses of ALI cultures and bronchial brushings

- Raw data files were analyzed using nSolver (version 4.0) per the software manual's instructions and data was passed through quality control steps, background correction, and normalized using housekeeping genes
- Pairwise differential expression was estimated and genes with p-value <0.05 were considered as significant for further enriched pathway analysis
- Significantly enriched pathways were identified using The Database for Annotation, Visualization and Integrated Discovery (DAVID; version 6.8)
- Targeted pathway mapping was used to identify genes associated with asthma-related cytokine signaling, and the compiled gene list was mapped to genes significantly regulated by TD-8236 in ALI cultures; similar expression trends between donors were identified

RESULTS

Reanalysis of public data set (GSE63142) demonstrates distinct and overlapping gene expression profiles based on asthma severity or FeNO level

Severity-based analyses

	Severe asthmatics vs healthy control	Log$_2$ fold change	Nonsevere asthmatics vs healthy control	Log$_2$ fold change
1	CEACAM5	1.76	CLCA1	2.01
2	PRR4	1.68	CPA3	1.86
3	TCN1	1.45	PRR4	1.78
4	CLCA1	1.43	TPSAB1	1.58
5	CXCL14	1.25	CEACAM5	1.39
6	NOS2	1.23	NOS2	1.26
7	TPRXL	1.21	SLC22A16	1.12
8	CPA3	1.14	ITLN1	1.09
9	FKBP5	1.13	TFF1	1.06
10	CD86	1.11	POSTN	1.00
11	TFF1	1.08	TFF3	0.93
12	KRT6A	1.07	TCN1	0.92
13	IL1R2	0.97	CST1	0.86
14	PYCR1	0.97	PYCR1	0.82
15	FGFBP1	0.97	MS4A2	0.81
16	UPK1B	0.96	UPK1B	0.81
17	AKR1B10	0.96	KIT	0.80
18	TFCP2L1	0.94	DHX35	0.78
19	PHACTR3	0.92	GSN	0.77
20	HMBOX1	0.92	PRB2	0.76

FeNO-based analyses

	FeNO >30 ppb vs healthy control	Log$_2$ fold change	FeNO <30 ppb vs healthy control	Log$_2$ fold change	Gene expression vs FeNO value (ppb)	Correlation coefficient
1	CLCA1	2.71	CEACAM5	1.20	NOS2	0.62
2	PRR4	2.33	CPA3	1.16	CCL26	0.62
3	CEACAM5	2.06	PRR4	1.02	CDH26	0.60
4	CPA3	2.02	TCN1	1.00	CST1	0.60
5	NOS2	1.92	TPSAB1	0.97	ZMAT4	0.59
6	TPSAB1	1.55	CXCL14	0.95	CST2	0.58
7	TFF1	1.51	FGFBP1	0.90	KYAT1	0.57
8	POSTN	1.36	TFF1	0.88	SERPINB10	0.57
9	CST1	1.34	TPRXL	0.84	FETUB	0.55
10	SLC22A16	1.33	AKR1B10	0.80	POSTN	0.54
11	TCN1	1.30	FKBP5	0.78	C8A	0.54
12	PYCR1	1.21	UPK1B	0.76	SERPINB2	0.54
13	TFF3	1.20	KRT6A	0.76	CISH	0.53
14	MUC5AC	1.20	PHACTR3	0.76	CLCA1	0.51
15	CD86	1.17	ALPL	0.69	ADCY4	0.51
16	PRB2	1.17	PYCR1	0.69	HS3ST4	0.50
17	PRB1	1.12	CEACAM7	0.67	SEC14L1	0.49
18	CST2	1.10	CEACAM6	0.65	SPEG	0.49
19	GSN	1.04	FAM83D	0.64	HDC	0.48
20	ASRGL1	1.04	PEX6	0.63	DOK1	0.47

Top upregulated genes in severe asthmatics

Top upregulated genes in nonsevere asthmatics

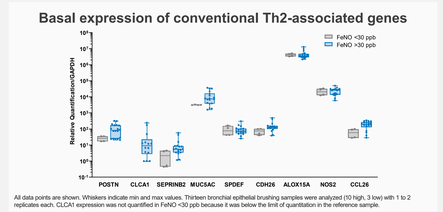
Top upregulated genes in both severe and nonsevere asthmatics

Genes of interest

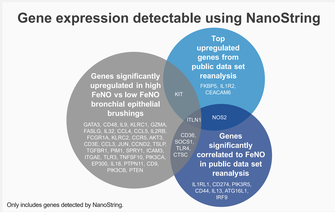
P <0.05 for all fold change values shown.

- NOS2 and CCL26 have the strongest correlation to FeNO, followed by CDH26
- NOS2 is detected in asthmatic populations regardless of disease severity
- Epithelial expression of POSTN, CLCA1, and SERPINB2 in the lung characterize T2 asthma as previously described[6]

Genes of interest are detected in bronchial epithelial brushings from asthmatic patients with high (>30 ppb) and low (<30 ppb) FeNO levels



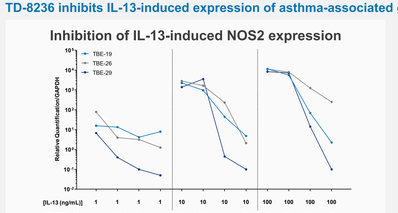
All data points are shown. Whiskers indicate min and max values. Thirteen bronchial epithelial brushing samples were analyzed (10 high, 3 low) with 1 to 2 replicates each. CLCA1 expression was not quantified in FeNO <30 ppb because it was below the limit of quantitation in the reference sample.



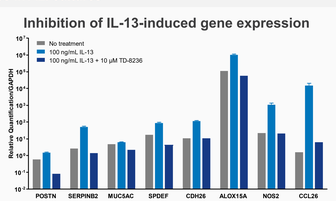
Only includes genes detected by NanoString.

- Detection of genes of interest was confirmed in bronchial epithelial brushing samples from mild asthmatic patients, independent of FeNO level
- The expression of many genes was upregulated in patients with high FeNO relative to those with low FeNO and was independent of asthma severity

TD-8236 inhibits IL-13-induced expression of asthma-associated genes in ALI cultures



Results from 3 donors are shown. ALI cultures were incubated with indicated agents for 8 days with media changed every 2 days. Each data point is the mean of 2 replicate ALI membranes with 1 PCR replicate each.



Mean and SD from one donor (TBE-19) are shown. ALI cultures were incubated with indicated agents for 4 hours. One ALI membrane per condition was tested. One PCR replicate was performed for no treatment and IL-13 + 10 μM TD-8236 conditions. Two PCR replicates were performed for 100 ng/mL IL-13.

- In ALI cultures, TD-8236 inhibited IL-13-induced NOS2 expression in a dose-dependent manner at multiple stimulation concentrations
- 10 μM TD-8236 also inhibited IL-13-induced expression of several other genes of interest related to asthma severity, FeNO, and mucus production

TD-8236 inhibits basal asthma-associated gene expression in ALI cultures

Upregulated gene Downregulated gene

Top 60 genes inhibited by 1 μM TD-8236 in ALI cultures

	Gene	Log$_2$ fold change		Gene	Log$_2$ fold change		Gene	Log$_2$ fold change
1	C1S	−6.81	21	IFIT2	−1.61	41	HLA-DRB3	−1.25
2	C1R	−5.01	22	LTB4R2	−1.56	42	IRF1	−1.24
3	IFITM1	−3.90	23	IKBKB	−1.56	43	TMEM173	−1.23
4	IL6	−3.61	24	KCNJ2	−1.51	44	HLA-DQB1	−1.23
5	CXCL10	−3.57	25	RORC	−1.50	45	MX1	−1.23
6	PDGFRA	−3.48	26	PDCD1LG2	−1.48	46	BLNK	−1.22
7	CCBP2	−2.88	27	CD14	−1.44	47	CD274	−1.21
8	CFB	−2.59	28	IL1R1	−1.44	48	CSF1	−1.18
9	CFI	−2.51	29	S100A8	−1.43	49	RUNX1	−1.16
10	IFI35	−2.40	30	SERPING1	−1.43	50	SOCS1	−1.16
11	CCL2	−2.23	31	HLA-DPB1	−1.43	51	PYCARD	−1.15
12	DPP4	−2.03	32	HLA-DPA1	−1.40	52	FCGRT	−1.14
13	CD36	−1.94	33	CCL22	−1.37	53	IRF7	−1.14
14	CXCL9	−1.86	34	FLT3LG	−1.36	54	HLA-DRB1	−1.13
15	CD40	−1.80	35	IL7	−1.36	55	HLA-DMA	−1.12
16	HLA-C	−1.79	36	PSMB10	−1.35	56	LAMP3	−1.12
17	IDO1	−1.77	37	TAP1	−1.28	57	MUC1	−1.12
18	SOCS3	−1.66	38	IRAK3	−1.27	58	ICAM1	−1.09
19	LTB4R	−1.66	39	GBP1	−1.26	59	LY96	−1.08
20	HLA-DQA1	−1.61	40	CD74	−1.25	60	STAT5A	−1.07

P <0.05 for all fold change values shown.

Genes upregulated in high FeNO vs low FeNO bronchial epithelial brushings are inhibited by TD-8236 in ALI cultures

Gene	High FeNO vs low FeNO bronchial epithelial brushings	1 μM TD-8236 vs vehicle
	Log$_2$ fold change	
CD36	1.63	−1.94
SOCS1	1.04	−1.16
TNFSF10	0.34	−0.79
CTSC	0.46	−0.67
TLR3	0.42	−0.55
GATA3	2.24	−0.38
JUN	0.94	−0.37
EP300	0.29	−0.19

P <0.05 for all fold change values shown.

Pathway analyses of genes inhibited by 1 μM TD-8236 in ALI cultures

Term	Resource	Accession	Gene count	P-value	Gene expression trend
Interferon-γ-mediated signaling pathway	Gene Ontology Biological Process	GO:0060333	18	0.00	HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DQA1, GBP1, STAT1, HLA-DQB1, PML, CD44, ICAM1, IFNGR1, HLA-B, IRF1, B2M, HLA-A, IRF7, HLA-DRB1, HLA-DRA
Antigen processing and presentation	Gene Ontology Biological Process	GO:0019882	13	0.00	HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DQA1, HLA-DQB1, HLA-B, HLA-DMB, FCGRT, CD74, HLA-A, HLA-DRB1, PSMB8, HLA-DRA
Graft-vs-host disease	KEGG Pathway	hsa05332	12	0.00	HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DQA1, HLA-DQB1, HLA-B, HLA-DMB, HLA-DMA, IL1A, HLA-A, HLA-DRB1, HLA-DRA
Asthma	KEGG Pathway	hsa05310	9	0.01	HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DQA1, HLA-DQB1, HLA-DMB, HLA-DMA, HLA-DRB1, HLA-DRA
Type I interferon signaling pathway	Gene Ontology Biological Process	GO:0060337	9	0.02	IFITM1, STAT1, IFNAR2, MX1, HLA-B, IRF1, HLA-A, IRF7, PSMB8
T cell receptor signaling pathway	Gene Ontology Biological Process	GO:0050852	11	0.04	HLA-DPA1, HLA-DPB1, HLA-DRB3, HLA-DQA1, HLA-DQB1, PSMB5, PSMB10, HLA-DRB1, PSMB9, PSMB8, HLA-DRA

- NanoString greatly expands the candidate gene pool from asthmatic bronchial epithelial cells grown in ALI sensitive to TD-8236 at baseline
- A subset of these genes that were upregulated in the FeNO >30 vs FeNO <30 ppb bronchial brushings are sensitive to TD-8236
- Pathway analysis on inhibition of baseline gene expression in asthmatic ALI cultures identifies asthma-relevant inflammatory processes

CONCLUSIONS

- Candidate genes relating to FeNO and asthma severity were identified from a public data set and many of the genes were expressed in bronchial epithelial brushings from asthmatic patients regardless of FeNO level
- TD-8236 inhibits many of the candidate FeNO-related and asthma-severity-related genes at baseline and with IL-13 induction in air-liquid interface cultures derived from asthmatic bronchial epithelial cells
- Many inflammatory processes relevant to asthma were identified by pathway analysis of TD-8236-inhibited gene expression in air-liquid interface cultures
- The broad effect of TD-8236 in air-liquid interface cultures demonstrates the potential to treat the heterogeneous inflammation associated with asthma

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References: 1. Kuruvilla ME, et al. *Clin Rev Allergy Immunol* 2019; 56: 219-33. 2. Modena BD, et al. *Am J Respir Crit Care* 2014; 190: 1363-72. 3. Wan XC, et al. *Immunol Allergy Clin North Am* 2016; 36(3): 547-57. 4. McNamara A, et al. *Eur Resp J* 2019; 54: Suppl. 63: OA4952. 5. Sana R, et al. *Eur Resp J* 2019; 54: Suppl. 63: PA3872. 6. Woodruff PG, et al. *Proc Natl Acad Sci USA* 2007; 104(40):15858-63.

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