

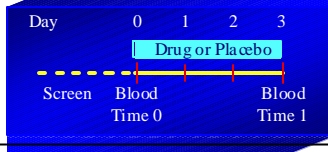
## Abstract

Corticosteroids are widely used anti-inflammatory drugs for the treatment of asthma, but are associated with side effects that limit their clinical usefulness. A better understanding of the effects of corticosteroids on cells and soluble factors involved in the pathogenesis of asthma should speed the development of beneficial drugs with fewer side effects. Toward this end phenotypic profiling was performed on 80 subjects divided into three main groups: 1) subjects with allergy and mild asthma; 2) subjects with allergy only and 3) healthy controls. Blood samples were analyzed before and after a three-day course of either prednisone or placebo treatment. A total of 725 unique measurements including 250 cell population counts, 488 cell surface antigen intensities, and 67 soluble factors were analyzed. A new microvolume laser scanning cytometry platform (SurroScan™) was used for the characterization of white blood cells. Plasma level of secreted mediators was determined by ELISA. Since multiple tests were performed to analyze this broad data set, the step-down Bonferroni p-value adjustment method of Holm was employed to guard against false positive conclusions.

There were many significant differences in the prednisone group, but not the placebo group (26% vs. 0.1% of the 725 variables). IgE levels increased while soluble CD23, the IgE Fc receptor, decreased post prednisone treatment. The acute phase protein, CRP, decreased, however SAA, another acute phase protein, increased post prednisone. The most significant change was seen in MMP-3 plasma level (5-fold increase post prednisone). For soluble cytokine receptors, IL-1 sR1 was increased and sTNF-R1 was decreased. There were also significant changes in circulating cells post treatment. Eosinophils decreased by half, while other granulocytes increased almost 2-fold. B cells and monocytes also increased significantly, while little change was seen in NK cells or total T cells. There were significant decreases in subpopulations of CD4 T cells, including those that express CD38 (involved in cell activation/adhesion) and CD45RA (naïve T cells). Expression levels of HLA class II molecules decreased significantly to 50% pre-drug levels on B cells and 60% on monocytes. It is hoped that this kind of phenotypic profiling will lead to a better understanding of the targets involved in glucocorticosteroid therapy and, in turn, to better treatment regimens.

## Phenotypic Profiling in Asthma/Allergy Study Design

- SurroMed proof of principle study
- 80 Subjects
  - asthma/allergy (mild asthma)
  - healthy controls
- Prednisone vs. placebo
  - oral, 20 mg qd
- Two blood



## Phenotypic Profiling in Asthma/Allergy Methodology

- Soluble factors: ELISA
  - Cytokines, chemokines, Ig, Acute phase proteins, MMPs, TIMPs, soluble receptors, soluble cell adhesion molecules.
- Cell populations and intensities: MLSC
  - Subsets of T cells, B cells, NK cells, Granulocytes, Eosinophils, Monocytes
  - Markers of activation, adhesion, costimulation, naïve/memory cells, HLA class II, etc.

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## Microvolume Laser Scanning Cytometry (MLSC)

Proprietary instrumentation, reagents, consumables and software for quantitation of cell populations in small volumes of whole or processed blood = integrated solution



- Uses combinations of fluorophore-tagged antibodies to cell surface markers
- 64 assays / 10 µL each > 200 populations
- Instrument control, data processing, uploading completely automated
- Yields absolute cell counts
- Designed, manufactured (12+), operated

## #A-E-030 Phenotypic Profiling in Asthma and Allergy: Effect of prednisone on cell surface and soluble factors

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## Prednisone affects levels of cells in blood (cell counts/ml)

Population	Trend	Mean Before Drug (N = 41)	Mean After Drug (N = 41)	Adjusted P-Value	Expected
B cells	-	260	406	<0.001	No
Granulocytes	-	3924	7394	<0.001	Yes
WBC	-	6852	10,346	<0.001	Yes
Eosinophils	-	175	65	0.009	Yes
Monocytes	-	369	478	0.02	No
NK cells	-	174	189	1	Yes
CD8 T cells	-	429	441	1	Yes
CD4 T cells	-	798	806	1	Yes
T cells	-	1268	1283	1	Yes
Lymphocytes	-	2382	2403	1	Yes

## Significant differences post prednisone

- Expected results based on literature
- Adjusted p-value < 0.05
- No differences observed for placebo group

Variable	Trend	Mean Pre Drug Cell population (cells/ml)	Mean Post Drug
WBC	-	6852	10,346
Grans.	-	3924	7394
Eosinophils	-	175	65
B Cells	-	260	406
Cell surface antigen (relative intensity)			
HLA-DR on monocytes	-	2966	1970
Soluble factor (concentration)			
CRP	-	2.70	1.16

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## Conclusions

- Robust data collection: 160 samples, 725 variables
- Big drug vs. placebo effect
  - Broad spectrum anti-inflammatory and immunosuppressive agent
- Significant differences observed in all types of bioanalytical measurements:
  - absolute cell counts
  - relative cell types
  - cell surface antigen expression
  - soluble factors.
- Some disease group differences
  - Total IgE higher in allergic subjects
  - Eosinophils higher in asthmatic subjects

## Prednisone

Analyte	Trend	Mean Pre Drug (SD)	Mean Post Drug (SD)	Adjusted P-value	Expected
IgE ng/ml	-	216 (309)	280 (368)	<0.001	Yes
sCD23 µg/ml	-	30.2 (17.8)	19.8 (12.4)	<0.001	Yes
IL-1aR2 ng/ml	-	8.16 (2.61)	10.10 (2.74)	<0.001	Yes
TNFR2 ng/ml	-	2.36 (1.14)	1.81 (1.02)	0.005	Yes
CRP µg/ml	-	2.70 (3)	1.16 (0.94)	0.008	Yes
SAA µg/ml	-	2.73 (3.3)	6.71 (14.5)	0.005	No
MMP-3 ng/ml	-	37 (30)	188 (132)	<0.001	No

## Significant differences post prednisone

- Novel or unexpected results
- Adjusted p-value < 0.05
- No differences observed for placebo group

Variable	Trend	Mean Pre Drug Cell population (cells/ml)	Mean Post Drug
Monocytes	-	369	478
Relative cell population (% of parent)			
CD89+ granulocytes	-	85	96
CD45RA+ CD4 T cells	-	48.8	36.4
Cell surface antigen (relative intensity)			
CD89 on granulocytes	-	2047	2527
HLA-DR on B Cells	-	8108	4486
HLA-DP on B Cells	-	9035	4743
HLA-DQ on B Cells	-	3123	1680
Soluble factor (concentration)			
MMP3	-	37	188
SAA	-	2.73	6.71

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