# Effects of Glucocorticosteroids on Cell Surface and Soluble Factors

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**will spearhead the development of beneficial drugs with fewer side effects. Biomarker discovery at SurroMed includes a wide range of assays to phenotype subjects with different disease subclasses and treatment regimens. The differential phenotyping tools include cytometry assays to analyze different cell types, immunoassays to quantitate soluble factors, and mass spectrometry to characterize proteins and small organic molecules. This phenotyping panel has been used to characterize biomarkers in a group of subjects with allergy and asthma undergoing a short course of glucocorticosteroid treatment. Cell surface and soluble factors have been analyzed from approximately 80 subjects from the following patient cohorts: 1) mild asthmatic, allergic (positive methacholine challenge, and a positive skin prick test to one or more tested allergens) 2) non-asthmatic, allergic (negative methacholine challenge, and a positive skin prick test to one or more tested allergens) 3) non-asthmatic, non-allergic (negative methacholine challenge and a negative skin prick test). Blood samples were analyzed both before and after a three day course of glucocorticosteroids or placebo taken twice a day.**

**A new microvolume laser scanning cytometry (MLSC) platform developed at SurroMed (SurroScan™) allows for the identification, characterization and enumeration of over 200 unique populations of cells, including subsets of lymphocytes, monocytes, neutrophils, eosinophils and NK cells from less than 2 ml of blood. In MLSC, a suspension of whole blood is incubated with a cocktail of fluorescently labeled antibodies specific for the relevant cell surface antigens, loaded onto capillary arrays and imaged with a confocal laser scanning microscope. In the current study a panel of 64 three-color assays was applied and both absolute cell counts of each population and level of antigen expression of each marker was analyzed. A significant increase in total numbers, and subpopulations of B cells, neutrophils, and monocytes was observed in the glucocorticosteroid treated group compared to placebo. A significant decrease in eosinophils was also observed post-treatment. Furthermore, differences in cell surface expression levels were detected, including significant decreases in the levels of HLA DP, DQ, and DR on both monocytes and B cells following glucocorticosteroid treatment.**

**The immunoassay panel includes detection of over 60 soluble factors by ELISA, including immunoglobulin subtypes, cytokines, chemokines and soluble receptors. These data are still being analyzed; differences between the different subject cohorts and treatment regimens have been identified. It is hoped that this kind of phenotyping data will lead to a better understanding of the targets involved in glucocorticosteroid therapy for allergy and asthma and, in turn, to better treatment regimens.**

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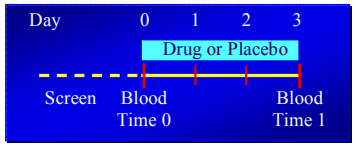
## Phenotypic Profiling in Asthma/Allergy Study Design

- SurroMed proof of principle study
- 80 Subjects
  - asthma/allergy (mild asthma), allergy, healthy controls
- Prednisone vs. placebo
  - oral, 20 mg, 2x/day
- Two blood samples per subject

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## Phenotypic Profiling in Asthma/Allergy Methodology

- 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.
- Soluble factors: ELISA
  - Cytokines, chemokines, Ig, Acute phase proteins, MMPs, TIMPs, RF, soluble cell adhesion molecules, 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**

## Phenotypic Profiling in Asthma/Allergy Statistics

- 725 unique variables
  - Cell populations: 250
  - Cell surface intensities: 408
  - Soluble factors: 67
- Conservative (step-down Bonferroni) method to protect against false positive errors
- Paired comparisons, pre & post treatment

**Significant Measures**

| Prednisone | Placebo |
|---|---|
| 188 | 1 |

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## 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 | Mean Post Drug |
|---|---|---|---|
| **Cell population (cells/µl)** | | | |
| 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

## Prednisone affects levels of cells in blood

| Population | Trend | Mean Before Drug (N = 39) | Mean After Drug (N = 39) | Adjusted P-Value | Expected |
|---|---|---|---|---|---|
| B cells | – | 276 | 283 | 1 | Yes |
| Granulocytes | – | 3989 | 3951 | 1 | Yes |
| WBC | – | 6949 | 6851 | 1 | Yes |
| Eosinophils | – | 81 | 81 | 1 | Yes |
| Monocytes | – | 365 | 367 | 1 | Yes |
| NK cells | – | 153 | 159 | 1 | Yes |
| CD8 T cells | – | 470 | 478 | 1 | Yes |
| CD4 T cells | – | 797 | 801 | 1 | Yes |
| T cells | – | 1269 | 1279 | 1 | Yes |
| Lymphocytes | – | 2358 | 2393 | 1 | Yes |

- Before vs. after
- Major cell types
- Counts per uL of blood

**Placebo**

**Drug**

| | Trend | Mean Before Drug (N = 41) | Mean After Drug (N = 41) | Adjusted P-Value | Expected |
|---|---|---|---|---|---|
| B cells | ↑ | 260 | 406 | <0.001 | Yes |
| Granulocytes | ↑ | 3924 | 7394 | <0.001 | Yes |
| WBC | ↑ | 6852 | 10,346 | <0.001 | Yes |
| Eosinophils | ↓ | 70 | 44 | 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 |

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## Significant differences post prednisone

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

| Variable | Trend | Mean Pre Drug | Mean Post Drug |
|---|---|---|---|
| **Cell population (cells/µl)** | | | |
| 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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