EFFICACY, SHORT TERM SAFETY AND SERUM BIOMARKERS IN A CLINICAL TRIAL OF IND#70190

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BACKGROUND

Asthma and chronic obstructive pulmonary disease (COPD) are known risk factors for lung cancer. Recent meta-analysis demonstrated increased risk of lung cancer in a population of never smoking individuals with asthma. The disease may be preventable, but many potential chemopreventive agents have not shown clinical activity in individuals at risk for lung cancer (Van Zandvijk et al, Lung Cancer 2003, 42:S71). Our open-label pilot trial evaluated efficacy, short-term safety and serum biomarkers of a novel natural product blend (IND#70190) designed to reduce asthma symptoms in a group of 32 men and women (18-80 yrs) with diagnosed and under-controlled asthma. Thirty-two men and women (age 18-80) with diagnosed and under-treated asthma received escalated doses on JJ03 in Stage I (single dose, observation at 2 weeks), Stage II (Stage I followed by additional two courses of weekly therapy and observation at week 4), and Stage III (Stage II followed by daily therapy for 4 weeks and observation at 8 weeks). The Stages I, II, and III were completed by 25, 23 and 11 subjects.

METHODS

- Endpoints measured at the baseline (week 1) and 2, 4, and 8 weeks of therapy included peak flow spirometry, nurse evaluation, medication scores, and energy assessment
- Peripheral blood serum specimens were obtained at the baseline and after drug treatment for 2, 4, and 8 weeks
- Liquid sample handling was performed in a Biomek 2000 robotic workstation (Beckman Coulter, Fullerton, CA)
- Serum levels of vascular endothelial growth factor (VEGF), matrix metalloproteinases MMP-2 and MMP-9, cyclic AMP (cAMP) and cyclic GMP (cGMP) were quantified by ELISA (R&D Systems, Minneapolis, MN)
- Serum proteins were analyzed in duplicate by surface enhanced ligand desorption/ionization time-of-flight mass spectrometry (SELDI TOF MS) on pre-activated IMAC-3 ProteinChip arrays and read in a Protein Biology System IIC (Ciphergen Biosystems, Fremont, CA)
- Statistical analyses were performed using paired t-tests for paired comparisons and general linear mixed-effect models for trends across assessment week
- SELDI data mining was done with Biomarker Wizard and CiphergenExpress software

SUMMARY

- There were no adverse clinical effects of the therapy
- The levels of VEGF and MMP-2 were not significantly affected in all patients
- Significant ($p<0.05$) inhibition of MMP-9 correlated with increased air flow
- Protein clusters associated with drug treatment were identified by SELDI

CONCLUSIONS

- The findings warrant identification and characterization of biomarkers for asthma and IND #70190 efficacy
- SELDI serum profiling is a feasible approach to investigating surrogate endpoints of drug efficacy in the clinic

RESULTS

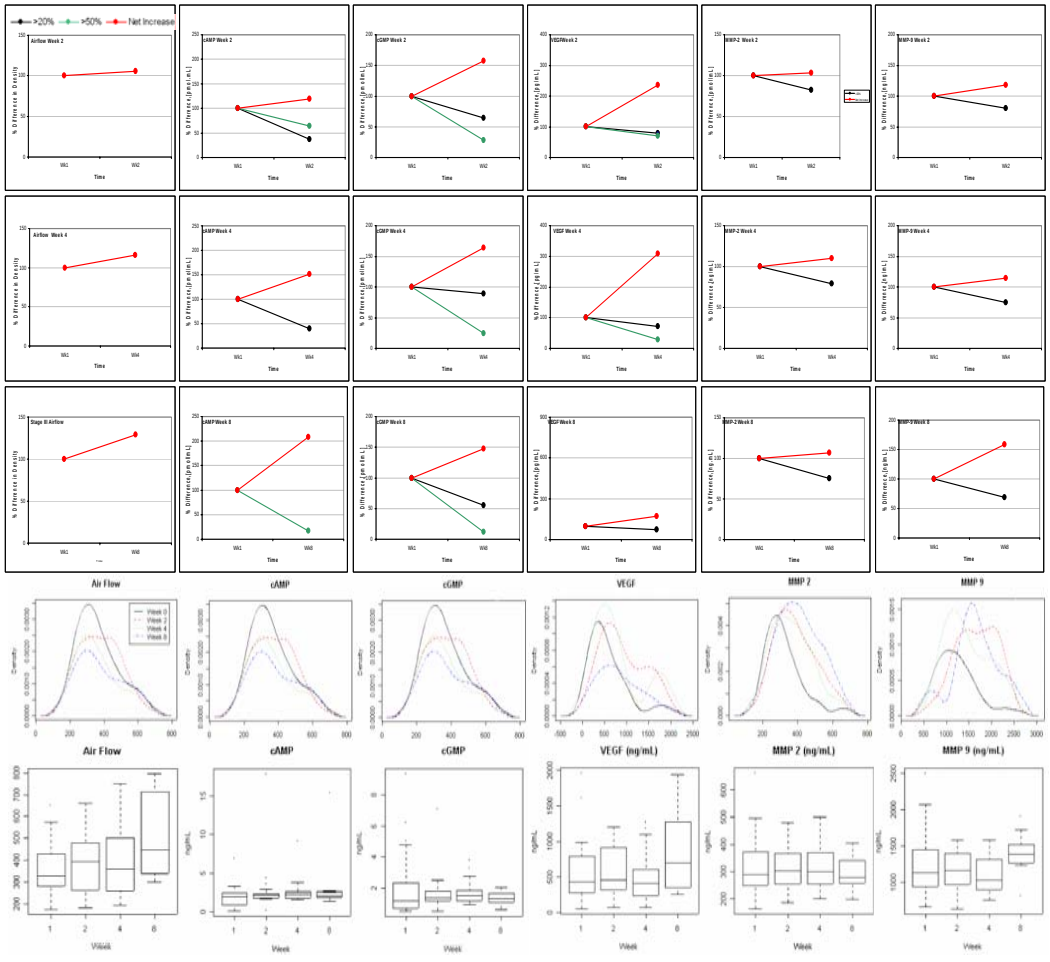

Figure 1 (top): Trendline graphs for air flow and serum biomarkers at Stage I (2 weeks), Stage II (4 weeks), and Stage III (8 weeks) in comparison with the baseline levels. Red; net increase, black; >20% inhibition, green; >50% inhibition.
Figure 2 (middle): Kernel density plots for air flow and serum biomarkers.
Figure 3 (bottom): Box plots of air flow and serum biomarkers at each week of data collection.

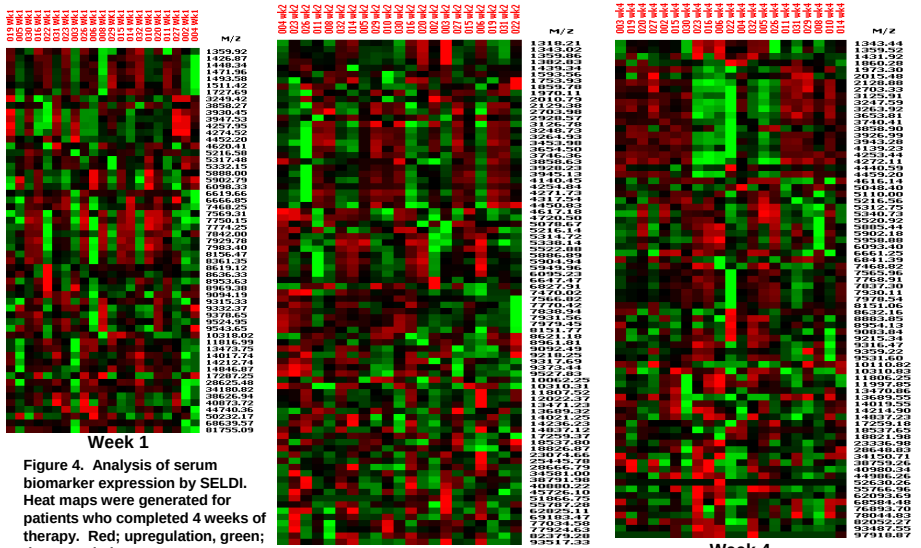

Figure 4. Analysis of serum biomarker expression by SELDI. Heat maps were generated for patients who completed 4 weeks of therapy. Red; upregulation, green; downregulation.

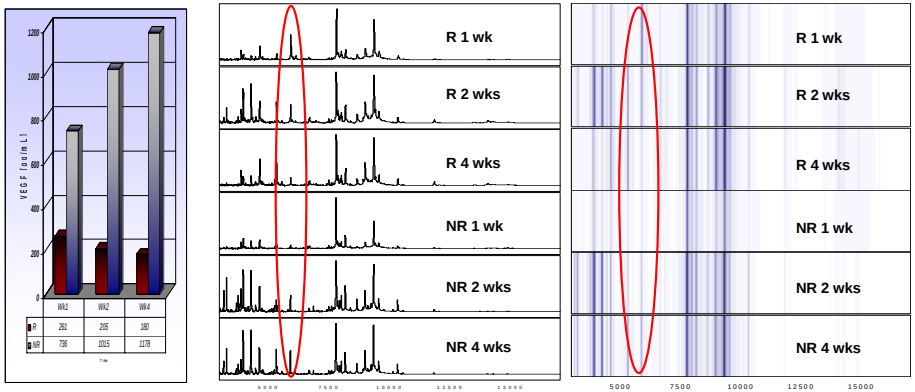

Figure 5. Comparison of serum protein profiles with VEGF levels. Protein profiles in a responder (R; significant inhibition of VEGF; three upper images) and non-responder (NR; VEGF levels increased over the course of therapy; three lower images) were analyzed at the baseline, at 2 weeks, and 4 weeks of therapy.