Enumeration of CECs and iNOS Expression Analysis in Patients with Type II Diabetes or Chronic Kidney Disease

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molecular profiling & diagnostics

Clinical Application: Pharmacodynamic Analysis of a Novel Treatment for Chronic Kidney Disease and Type 2 Diabetes

Key Words: CECs, diabetes, chronic kidney disease, biomarker expression

Background: Multiple studies in chronic kidney disease (CKD) have illustrated associations between measures of inflammation and reduced kidney function; however, to date, few treatments that inhibit general inflammatory processes have been examined as potential options for CKD or type 2 diabetes (T2D) treatments. This trial examined one such agent, bardoxolone methyl, and tracked multiple biological endpoints to measure the treatment's efficacy. ApoCell was contracted to monitor the levels of endothelial dysfunction and vascular injury by analyzing the number of circulating endothelial cells (CECs), and the inflammatory status of the CECs by evaluating the inducible nitric oxide synthase (iNOS) levels present in each CEC.

Methods: Blood samples were drawn from twenty patients at two timepoints – baseline and day 56 – across two clinical sites, and shipped to ApoCell for processing. ApoCell tested the patient samples using the CellSearch® CEC kit, enriching for CD146+ cells, and staining for DAPI, CD105, CD45, and the biomarker of interest, iNOS. Samples were then analyzed on the CellTracks® Analyzer II by an ApoCell scientist, a Veridex Certified Analyist. CECs were characterized as CD146+/DAPI+/CD105+/CD45- cells, and the presence or absence of iNOS was also evaluated on each identified CEC.

Results:

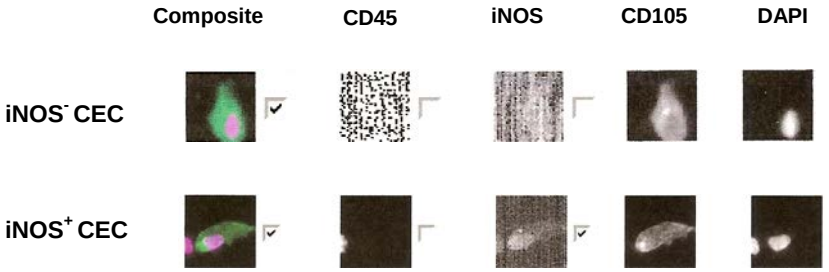

Figure 1. Representative Images of CECs. Gallery images used for identification of CECs on the CellTracks® Analyzer II. Two CECs are shown here; one that is negative (top row) for iNOS (third column), and one that is positive (bottom row) for iNOS. Both rows exhibit the DAPI+/CD105+/CD45- phenotype that is classified as a CEC.

	Baseline	Day 56	Change[a]	P Value[b]
Circulating endothelial cells, CD105+/ml[c]	5.3 ± 3.0	3.5 ± 1.7	- 1.9 ± 2.5	0.007†
Circulating endothelial cells, CD105+ and iNOS+ /ml[c]	2.9 ± 1.4	1.0 ± 1.0	- 1.9 ± 1.8	0.001†

Figure 2. Summary of CEC markers at baseline and change at day 56. [a] Day 56 minus baseline; [b] p values calculated from two-sided paired t tests; † Indicates statistically significant change from baseline; [c] n=16 for baseline, n=17 for day 56, n=16 for change.

Treatment with bardoxolone methyl significantly decreased both the total number of CECs and the number of iNOS positive CECs as compared to baseline. The total counts were depressed by 28%, whereas the count of iNOS positive CECs was decreased by 46%.

Impact: Bardoxolone methyl shows promise as a potential treatment for patients with T2D or CKD, and ApoCell's findings corroborated the trends found in the other biological endpoints that were examined in this study. Increased CECs have been directly linked to endothelial dysfunction and renal disease as well as hypertension. The decreases in CECs and iNOS+ CECs indicate that the drug has the potential for reduced vascular damage and improved inflammatory status within the vasculature, as indicated by the larger reduction in the number of CECs that stained positively for iNOS. Further study is necessary to evaluate the full effects of treatment, but initial findings illustrate that this compound represents a strong potential treatment for a disease that afflicts many.

References: 1. Pergola, PE, et al. "Effect of Bardoxolone Methyl on Kidney Function in Patients with T2D and Stage 3b-4 CKD." Am J Nephrol 2011; 33: 469-476.

ApoCell, Inc.
2575 West Bellfort, Suite 190 | Houston, TX 77054
Office: (713) 440-6070 | Fax: (713) 440-6074
biomarkers@apocell.com | www.apocell.com