Prospective Real-time Analysis of P-cadherin Expression to Select Patients into a Phase I Oncology Trial

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Introduction

- P-cadherin, a Ca^{2+}-dependent cellular adhesion protein, belongs to the family of classic cadherins that are engaged in various cellular activities including motility, adhesion, invasion, and signaling of tumor cells[1].
- P-cadherin is strongly overexpressed in esophageal, gastric, pancreatic, bladder, and breast cancers.
- P-cadherin is thought to contribute to the oncogenesis of many types of cancers, including breast cancer, colorectal cancer, and head and neck cancers.
- Downregulation of E-cadherin expression with concomitant upregulation of N-cadherin or P-cadherin, termed cadherin switching, has been reported for carcinomas of the esophagus, prostate, cervix, and ovary, and has been associated with tumor progression and metastatic disease.
- P-cadherin may be a suitable target for therapeutic intervention in cancer patients whose tumors aberrantly express P-cadherin.

Objectives

P-cadherin (placental cadherin) is a Ca^{2+}-dependent cell-cell adhesion integral membrane glycoprotein that plays a role in the maintenance of the epithelial structure. This objectives of this immunohisto-chemical (IHC) study were to determine P-cadherin expression levels in the archival biopsy of cancer patient with solid tumors as a criterion for patient enrollment and to identify a range of expression to correlate with clinical outcome in an ongoing Phase I trial.

Patients and Methods

Archival tumor biopsies were obtained from 94 different advanced solid cancer patients who were eligible for enrollment at 3 global trial sites. Validated IHC staining kits for P-cadherin detection were distributed to the trial sites for on-site staining. Cross-validation of the IHC scoring and staining was performed in a masked fashion at ApoCell using a board-certified pathologist. An IHC scoring index (SI = area x intensity) was used (**Table 1**). A SI of ≥ 4 was required for inclusion. In addition, P-cadherin was detected by immunofluorescence (IF) for laser scanning cytometry (LSC)-mediated quantitative analysis.

Results

Table 1. IHC Scoring Index of Area and Staining Intensity for P-cadherin Staining

Area (Positive for P-cadherin)	Score	Staining Intensity	Score
0	0	None	0
< 20%	1	Low	1
20 - 50%	2	Moderate	2
> 50%	3	High	3

Criteria for Patient Enrollment (Scoring Index [SI] ≥ 4 Required for Inclusion)

Included Patients:
Any patient with ≥ 20% Area and ≥ Moderate Intensity: SI≥ 4
A patient with 20-50 % Area (2) and > Moderate Intensity (2): SI= 4
A patient with > 50% Area (3) and Moderate Intensity (2): SI= 6
A patient with > 50% Area (3) and High Intensity (3): SI= 9

Excluded Patients:
Any patient with < 20% Area and ≤ Low Intensity: SI= 0-1
A patient with 20-50% Area (2) and > Low Intensity (1): SI= 2
A patient with < 20% Area (1) and > High Intensity (3): SI= 3

- Expression of P-cadherin was determined by immunofluorescence and quantified by laser
- scanning cytometry (LSC).

Study Design

- Each site ran independent IHC assays and analyses by board-certified pathologists, and provide stained and unstained archival biopsy slides for independent, masked analysis by ApoCell Inc (Houston, TX).
- Advanced solid cancer patients with a P-cadherin positive archival biopsy were deemed eligible for enrollment into an ongoing trial. Other clinical eligibility criteria were also applied. Clinical results will be reported separately.
- P-cadherin positivity was defined by a scoring index (SI) [product of the tumor area positive for P-cadherin x staining intensity]. A SI ≥ 4 is associated with poor clinical outcome[2] and was chosen as one criterion for patient eligibility into the trial.
- Immunofluorescence assay of P-cadherin and LSC-mediated quantitative analysis were performed by ApoCell.

Table 2. Summary of P-cadherin IHC Data

	Trial Site	Number of Patients	Eligible	Excluded	Discrepancy*
	USA	14	10	4	1
	Australia	42	31	11	4
	Korea	38	24	14	3
Total		94	65	29	8

*Discrepancy means that ApoCell determined a different result than the clinical site. There are 8 discrepancies total and the discrepancy rate is 8.5% (8 out of 94 patients).

Figure 1. P-cadherin IHC Scoring Summary
Scores obtained by ApoCell are plotted

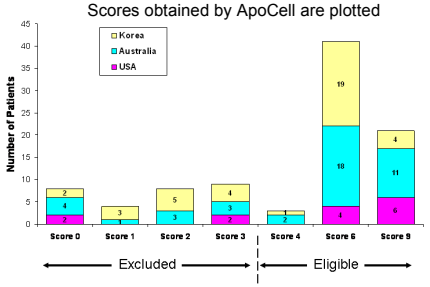
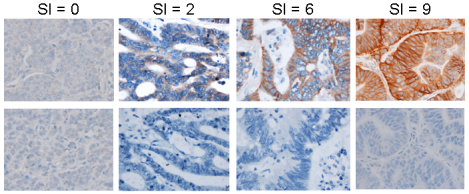
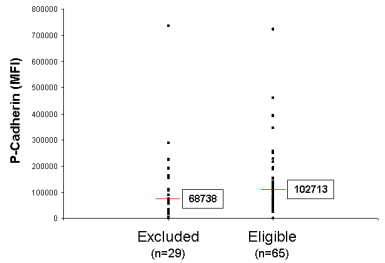


Figure 2. Representative IHC Images of Scoring Index (SI)



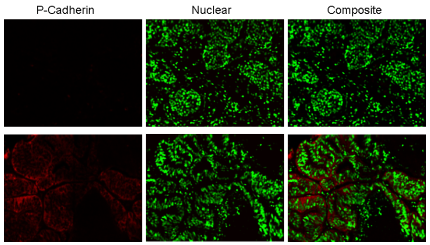
Top panel: P-cadherin staining. Bottom panel: negative control.

Figure 3: Dot Plot Display of Mean Fluorescence Intensity (MFI) of P-cadherin Acquired by LSC



The lines stand for the median of the subgroup. The median intensity of P-cadherin staining was 49.4% higher in the eligible versus excluded patient groups.

Figure 4. Representative Images of P-cadherin Staining by LSC



Top panel: negative control. Bottom panel: P-cadherin staining.

Conclusions

- Sixty-five of 94 Patients (69.2%) were found to be P-cadherin positive by the central reader, ApoCell. Median scoring index for all patients was 6 [mean SI = 5.3 ± 2.8 (SD)], range SI: 0 to 9).
- Good agreement in IHC scoring index was achieved between ApoCell and clinical sites (91.5% concordance rate: 86 of 94 patients).
- Successful execution of this IHC study demonstrates the feasibility of prospectively selecting patients based on IHC expression of P-cadherin using a custom standardized scoring index analysis across multiple clinical sites.
- LSC analysis is being evaluated in parallel to IHC for greater sensitivity of P-cadherin expression and correlation with clinical outcome.

References

1. Arnes JB, et al. Clin Cancer Res. 2005; 11:4003-11
2. Paredes J, et al. Breast Cancer Res. 2007; 9(5): 214

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