CEACAM6 is a candidate biomarker for pelareorep resistance in pancreatic adenocarcinoma (PDAC)

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Abstract

Background: Pelareorep is a proprietary formulation of live, replication-competent, naturally occurring Reovirus Type 3 Dearing strain. A randomized phase II trial of pelareorep in combination with carboplatin and paclitaxel in first-line treatment of metastatic PDAC (NCT01280058) was performed. Although pelareorep did not improve the primary endpoint of progression-free survival compared to carboplatin and paclitaxel alone, impressive durable responses were seen in the pelareorep arm in some patients. Further, prior studies have noted the immunomodulatory CEA cell adhesion molecule 6 (CEACAM6/CD66c) as a receptor for specific viral subtypes. We thus speculated that altered CEACAM6 levels may be predictive for pelareorep sensitivity and may serve as a biomarker.

Methods: 73 patients were enrolled on this randomized phase II trial of pelareorep in combination with carboplatin and paclitaxel (Arm A) versus carboplatin and paclitaxel alone (Arm B). Pre-treatment tissue biopsies were collected prior enrolment for all 73 pts on study. However, mRNA expression was available for only 31 pts, 17 on Arm A and 14 on Arm B. RNA was purified from FFPE tissue and gene expression analysis was performed using SensationPlus™ FFPE Amplification and WT labelling kit and the Human Transcriptome Array 2.0. The raw data was RMA normalized at the gene level and log2 transformed with the Affymetrix Expression Console. The Affymetrix Transcriptome Analysis Console 2.0 was used to perform differential gene expression analysis. Given the small data sets and the hypothesis generating nature of this analysis, the nominal p-value was used. Appropriate corrections for multiplicity were performed. Carcinoembryonic antigen-related cell adhesion molecule (CEACAM6) protein expression was determined by immunohistochemistry using antibody ab78029 (Abcam Inc., Cambridge, MA, USA) on a Leica BondRX autostainer. Immunohistochemical staining in luminal membrane and cytoplasm was scored by three independent readers who were blinded to clinical outcomes (CT, MJ, WF), on two occasions with discrepancies settled by discussion with the third reader (WF). Two of the readers (MJ and WF) were board certified pathologists.

Results: Low levels of CEACAM6 mRNA expression were associated with prolonged PFS in pelareorep-treated pts (p=0.05). There was no relationship between CEACAM6 mRNA levels and response in either arm (p=0.34). The luminal, but not the cytoplasmic immunohistochemistry score, was highly correlated with mRNA expression levels of CEACAM6, p=0.001.

Conclusions: Elevated levels of CEACAM6 are known to prevent entry of adenovirus into cells[2]. CEACAM6 may be included as a candidate biomarker of resistance to pelareorep and, in theory, could inhibit viral trafficking in tumor cells

Results

To identify potential biomarkers of response, we evaluated mRNA expression profiles of long-term responders (patients who received ≥10 cycles) as compared to patients who progressed early (received ≤5 cycles). Pre-treatment mRNA expression was available for 31 of the 73 patients enrolled in the study, 17 on Arm A and 14 on Arm B. Since this marker was not available on most of the subjects treated on this study, we evaluated if there were differences between those who did vs did not have CEACAM6 expression data analysed (**Table 1**).

CEACAM6 was the most differentially expressed gene in the pelareorep set of differentially expressed genes with an eight-fold decrease in levels in long-term responders as compared to early progressors.

Characteristic	No CEACAM6 data	CEACAM6 data	p-value
Arm A B	 19 23	 17 14	0.48
Gender F M	 19 23	 13 18	0.82
Age (years) Median (range)	64.5 (39 to 84)	64 (44 to 81)	0.75
PR No Yes	 36 6	 23 8	0.24
DCR No Yes	 23 19	 8 23	0.017
PFS (months) Median (95% CI)	3.76 (2.2, 5.8)	6.21 (4.5, 8.3)	0.026
OS (months) Median (95% CI)	7.13 (4.5, 11.2)	10.38 (7, 16.5)	0.07

Low CEACAM6 mRNA expression was associated with longer PFS in pelareorep arm

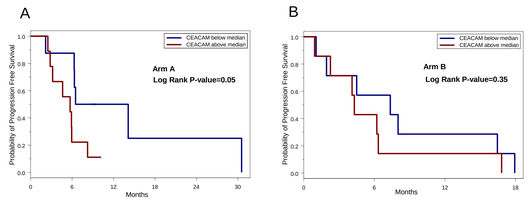

Figure 1. PFS was statistically significantly longer in patients with low mRNA expression of CEACAM6 in Arm A, n=17, p=0.05, (A) but no difference was seen in Arm B, n=14, p= 0.35,(B).

	PFS Median (95% CI)	Log-rank test
Arm A (n=17) (median CEACAM=5.24) CEACAM ≥5.24 CEACAM <5.24	 5.72 (2.50-8.25) 10.32 (2.17-30.55)	0.05
Arm B (n=14) (median CEACAM=4.73) CEACAM ≥4.73 CEACAM <4.73	 4.30 (0.92-6.34) 7.36 (1.05-16.46)	0.35

Table 2. Median PFS per arm of study dichotomized by high versus low CEACAM6 mRNA expression.

CEACAM6 mRNA	Hazard Ratio for Progression (95% CI)	LR P-value
Arm A	1.54 (1.12-2.13)	0.01
Arm B	1.07 (0.72-1.59)	0.74

Table 3. Hazard ratio for progression based on CEACAM6 mRNA expression levels. Data were calculated using the univariate Cox model with the continuous scale of CEACAM6. In Arm A, CEACAM6 mRNA expression level was very influential with HR=1.54, suggesting that one unit increase in CEACAM6, corresponds to an increase in the risk of progression and/or death by 54% in this arm (p=0.01). There was no significant relationship seen in Arm B. There was no relationship between CEACAM6 mRNA levels and response in either arm (p=0.34).

Luminal CEACAM6 levels correlated with CEACAM6 mRNA expression

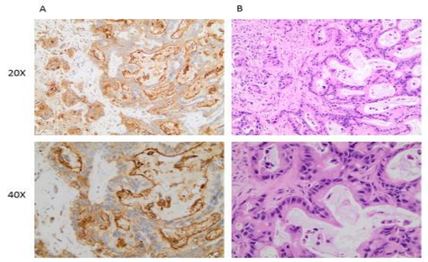

Figure 2. Representative image of CEACAM6 IHC. CEACAM6 staining as detected by ab78029 was defined as positive when staining was observed along the luminal cell membrane border or in the cytoplasm in ≥5% of cells in the tissue section (primary tumour or metastatic site). Intensity was scored as 0 if negative, 1+ if mild to moderate, 2+ if marked. Percentage tumour was scored as 0 if <5%, 1 if 5-35%, 2 if 35-69% and 3 if ≥70% of tumour cells stained positive. A quick score[1] was calculated for luminal and cytoplasmic staining by multiplying the intensity by the percentage tumour cells. Panel A demonstrates marked luminal membrane staining and cytoplasmic staining in a biopsy of pancreas from a patient on trial. Panel B represents the haematoxylin and eosin stained slides demonstrating morphology. Two magnification views are shown 20X (top panel) and 40X (bottom panel).

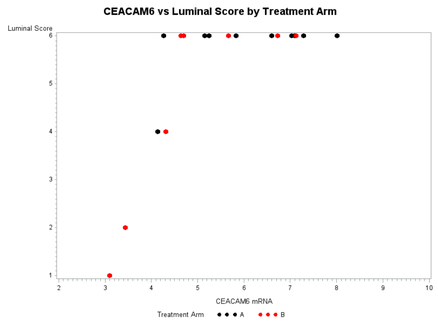

Figure 3. CEACAM6 vs. Luminal Score by Treatment Arm (Spearman correlation p-value=0.001). The number of patients with samples available for luminal score was 18, with 10 on Arm A and 8 on Arm B, and for the cytoplasmic score was 20, with 12 on Arm A and 8 on Arm B. The luminal, but not the cytoplasmic score, was highly correlated with mRNA expression levels of CEACAM6, p=0.001. There was no relationship between luminal CEACAM6 score and PFS.

Discussion

Overall, those who had CEACAM6 expression data available tended to be those patients who achieved better outcomes; therefore, caution should be applied in how generalizable any analyses are to the overall trial cohort.

CEACAM6, an immunoglobulin superfamily member, is differentially expressed in pancreatic adenocarcinoma cells and, when overexpressed, is associated with a higher level of anchorage-independent growth, increased invasion, and metastases[3-5]. CEACAM6 is a poor prognostic marker in KRAS mutant and wild type pancreatic ductal adenocarcinoma. In our study, low levels of CEACAM6 mRNA expression were associated with longer PFS in the pelareorep arm (ARM A) but not in the control arm (ARM B).

Previous studies have shown that CEACAM6 inhibits adenovirus infectivity of PCA cells by antagonizing SRC signalling. Through alteration in the distribution of and reduction in the expression of cytoskeletal proteins, such as tubulin and dynactin, CEACAM6 blocked adenovirus cytoplasmic trafficking to the nucleus[6].

We hypothesize that a similar mechanism underlies the resistance to pelareorep seen in our study in patients with high mRNA expression of CEACAM6. At the protein level, higher levels of luminal membrane expression of CEACAM6 were seen than cytoplasmic levels, and luminal membrane staining correlated with mRNA expression levels. Our numbers were very limited for immunohistochemistry and mRNA, but we suggest that further exploration of the relationship between CEACAM6 and pelareorep activity is warranted.

Conclusion

CEACAM6 may be included as a candidate biomarker of resistance to pelareorep and, in theory, could inhibit viral trafficking in tumor cells

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