ABSTRACT #921

Level of Expression of EpCAM and Response to Vicinium in Non Muscle-Invasive Transitional Cell Carcinoma of the Bladder

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BACKGROUND

The epithelial cell adhesion molecule EpCAM is a type I transmembrane glycoprotein that has limited normal expression on the basolateral surface of a variety of adult epithelial cells. EpCAM appears to be over-expressed in the majority of human epithelial carcinomas and EpCAM expression levels correlate with proliferative activity and neoplastic transformation. Vicinium™ is a recombinant fusion protein comprised of a humanized scFv specific for EpCAM and a truncated fragment of *Pseudomonas* exotoxin A (ETA 252-608) that is being developed as a treatment for non muscle-invasive TCC. In order to identify patients suitable for Vicnium treatment, a semi-quantitative immunohistochemical (IHC) clinical trial assay was developed for the determination of EpCAM expression in tumor tissues.

OBJECTIVE

The primary objective of the study was to determine the frequency and level of EpCAM expression on bladder carcinomas and to correlate those results with response to Vicinium treatment.

MATERIALS AND METHODS

IHC was performed on formalin-fixed, paraffin-embedded tissue specimens obtained from patients with CIS, Ta or T1 tumors being screened for entry into Vicinium clinical studies. After de-paraffinization and re-hydration, the slides were treated for antigen retrieval and then incubated with Vicinium followed with a rabbit polyclonal anti-ETA. Bound Vicinium/anti-ETA complex was detected using anti-rabbit polyclonal EnVision+HRP. Localization of the drug was visualized by the application of diaminobenzidine. Membrane staining intensity was assessed under light microscopy and graded on a 4 point scale with 0 being negative and 3+ being very strong. An overall EpCAM score from 0 to 3+ was assigned based on the maximum staining intensity observed in ≥ 1% of cells. EpCAM positive and negative controls (human colon carcinoma and human normal heart, respectively) were included in each staining. Forty five evaluable patients with EpCAM positive BCG refractory carcinoma in situ (CIS) of the bladder were enrolled into a Phase II Vicinium trial. Patients were treated with either 6 or 12 weekly instillations of Vicinium followed by 3 week maintenance courses administered every 3 months. Patients were assessed for a clinical response at 3, 6, 9 and 12 months by standard cystoscopy, cytology and biopsy. EpCAM expression as measured by IHC and response to Vicinium treatment were examined in these patients.

RESULTS:

Table 1

EpCAM Score	Number of Patients with Tumor (N=133)
0	1 (<1.0%)
1+	7 (5.3%)
2+	36 (27.0%)
3+	89 (66.9%)

Table 2

Stage	EpCAM Score		
	1+	2+	3+
Ta (n=33)	3 (9.1%)	12 (36.4%)	18 (54.5%)
T1(n=17)	1 (5.9%)	6 (35.3%)	10 (58.8%)
CIS(n=56)	0 (0%)	10 (17.9%)	46 (82.1%)
TOTALS (N=106)	4 (3.8%)	28 (26.4%)	74 (69.8%)

Table 3

Grade	EpCAM Score		
	1+	2+	3+
Grade 1*(n=1)	0 (0%)	0 (0%)	1 (100%)
Grade 2 (n=34)	3 (8.8%)	15 (44.1%)	16 (47.1%)
Grade 3 (n=15)	1 (6.7%)	3 (20.0%)	11 (73.3%)
CIS (n=56)	0 (0%)	10 (17.9%)	46 (82.1%)
TOTALS (N=106)	4 (3.8%)	28 (26.4%)	74 (69.8%)

*Grade 1 patients were not eligible for Vicinium studies

Bladder biopsies from 135 patients with CIS, TaG2-3 or T1G2-3, who were screened for enrolment into Vicinium clinical trials, were stained for EpCAM. Of these, 132 (98%) were positive and 3 (2%) were negative for EpCAM staining. A review of the 3 EpCAM negative biopsies revealed that there was no tumor present on the tissue section submitted for EpCAM staining for 2 of the biopsies while CIS was confirmed on the third EpCAM negative biopsy. EpCAM membrane staining was observed on cells distributed throughout the depth of the tumors. Of the positive tumors, the staining intensity for EpCAM was found to be 1+ on 7 (5.3%), 2+ on 36 (27.0%) and 3+ on 89 (66.9%) of the tissue specimens [Table 1]. The percentage of cells exhibiting membrane staining in the EpCAM positive biopsies ranged between 5% and 100% with most (78.6%) of the biopsies having from 50% to 100% of cells stained. The tumor stage and grade of the biopsy submitted for EpCAM staining could be confirmed for 106 of the biopsies. Most of the patients screened had CIS, of which the far majority (82.1%) demonstrated 3+ EpCAM staining [Table 2]. Fewer patients with Ta or T1 tumors were screened. Intense (3+) EpCAM staining was observed in 54.5% of Ta tumors and in 58.8% of T1 tumors [Table 2]. The frequency of intense (3+) EpCAM staining was significantly higher in CIS vs. Ta tumors (P = 0.0073)* and in high grade [CIS + G3] vs. lower grade tumors [G2 +G1] (P = 0.0014)*[Table 3]. Since only a single well differentiated (G1) tumor was examined in this study, no assessment can be made specifically for this tumor grade.

*Fisher's Exact Test

Bladder biopsies from the 45 patients with BCG refractory CIS treated with Vicinium in a Phase II study, revealed overall EpCAM scores that ranged from 1+ on 2 (4.4%), 2+ on 9 (20%) and 3+ on 34 (75.6%) of the tissue specimens [Table 4]. Of these 45 patients, 20 (44%) achieved a complete response at 3 or 6 months post treatment. Patients with all levels of overall EpCAM scores, which are based on staining intensity, were able to achieve complete responses. The ability to achieve a complete response was similar for patients with 2+ (44.4%) or 3+ (44.1%) EpCAM scores. Of the 2 patients with 1+ EpCAM scores, one was a complete responder. There was no significant difference in the ability to achieve a complete response when examined by either overall EpCAM scores [Table 4] or when further examined according to the percentage of cells exhibiting very strong (3+) EpCAM staining [Table 5]. Complete responders were also analyzed according to the percentage of total cells demonstrating any level of positive EpCAM staining [Table 6]. While there may be a trend suggesting that patients with biopsies that exhibit a greater percentage of EpCAM positive cells may have an increased chance of achieving a complete response to Vicinium treatment, the differences were not significant, likely due to the relatively small numbers of patients. The complete responses achieved, were durable in a subset of patients, with 17 % of treated patients maintaining a complete response up to at least 12 months (data not shown). The small numbers of patients treated in this study did not permit any definite conclusions regarding a correlation of EpCAM expression as measured by IHC, with the ability to maintain a durable complete response.

Table 4: CIS Patients in Phase II Study

	Overall EpCAM Score			
	1+	2+	3+	Total
All Patients	2/45(4.4%)	9/45 (20.0)	34/45 (75.6%)	45/45 (100%)
Responders	1/2 (50.0%)	4/9 (44.4%)	15/34 (44.1%)	20/45 (44.4%)

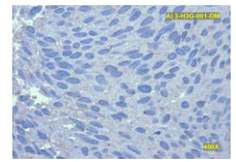
Table 5: CIS Patients in Phase II Study

% of 3+ Cells Stained	Responders at anytime
100%	2/2 (100%)
70 – 99%	7/17 (41.2%)
40 - 69%	5/8 (62.5%)
10 – 39%	1/7 (14.3%)
<10% (all 0s)	5/11 (45.5%)

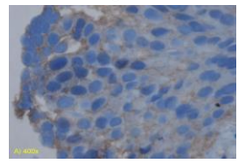
Table 6: CIS Patients in Phase II Study

% of Total Cells Stained	Responders at anytime
100%	9/17 (52.9%)
70 – 99%	8/21 (38.1%)
40 - 69%	1/4 (25.0%)
10 – 39%	2/2 (100%)
<10%	0/1 (0%)

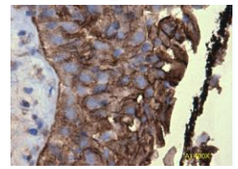
EpCAM Scoring of Biopsies from Patients with Non Muscle-Invasive TCC of the Bladder
Vicinium + Rabbit anti-PE + EnVision (400x)



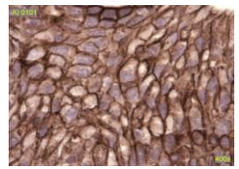
0+



1+



2+



3+

CONCLUSIONS:

- EpCAM is expressed in almost all non muscle-invasive TCC tumors.
- High levels of EpCAM expression are observed in most high grade tumors including CIS
- CIS patients with all levels of overall EpCAM scores,which are based on staining intensity, were able to achieve complete responses.
- There may be a trend suggesting that CIS patients with a greater percentage of EpCAM positive cells observed on biopsy, may have an increased chance of achieving a complete response to Vicinium treatment.
- Further study of the effect of EpCAM expression on response to Vicinium treatment is needed.
- Mutiple factors, in addition to the level of EpCAM expression, may likely have an impact on the response to Vicinium treatment.