LEVEL OF EXPRESSION OF EpCAM AND RESPONSE TO VICINIUM™ IN NON MUSCLE-INVASIVE TRANSITIONAL CELL CARCINOMA OF THE BLADDER

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ABSTRACT

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 single chain variable fragment (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 transitional cell carcinoma (TCC). In order to identify patients suitable for Vicinium 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 carcinoma in situ (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 $\geq$ 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 CIS of the bladder were enrolled into a Phase II Vicinium trial. Patients were treated with either 6 or 12 weekly instillations of Vicinium. This was 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.

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. Multiple factors, in addition to the level of EpCAM expression, may likely have an impact on the response to Vicinium treatment.