Use of HPV16 Circulating Tumor DNA Detected in Liquid Biopsies to Predict Response in Patients with Advanced HPV16-Positive Cervical Cancer

Paula Bousquet, Milena Blaga, Berit Nicolasen, Karsten Bruuns Slot, Agnete Fredriksen, Karoline Schjetne.* Nykode Therapeutics, Oslo, Norway

**Background** Circulating tumor DNA (ctDNA) can potentially provide a valuable tumor-specific and non-invasive biomarker for longitudinal monitoring of a patient’s response to therapy. We aimed to quantify HPV16 ctDNA in patients with advanced cervical cancer and explore the potential use of ctDNA to predict on treatment clinical responses to VB10.16 in combination with atezolizumab.

**Methods** This open-label, single-arm, Phase 2a trial was conducted in patients with HPV16-positive recurrent or metastatic cervical cancer. Patients received multiple doses of the therapeutic DNA vaccine VB10.16 in combination with the PD-L1 inhibitor atezolizumab.

Blood specimens were collected at baseline and every 9 weeks during treatment to quantitatively determine the HPV16 E7 viral DNA in plasma by duplex digital PCR (dPCR).

The primary endpoint of the trial was the objective response rate assessed by an independent central review using RECIST version 1.1 criteria. HPV16 ctDNA was correlated with the defined clinical response as an exploratory endpoint. The study was approved by the national regulatory authorities and Independent Ethic Committees (NCT04405349).

**Results** Of the 39 patients included in the efficacy population of this interim analysis, 21 had detectable HPV16 ctDNA samples at baseline. The presence of ctDNA at baseline was not associated with clinical benefit. However, early reduction of HPV16 ctDNA after treatment start correlated with clinical response and prolonged progression-free survival (p < 0.0021). Patients who had a clearance of HPV ctDNA to levels below the detection limit, all achieved some level of clinical benefit (DCR 100%). In contrast, on-treatment increases in HPV16 ctDNA was associated with poor clinical outcomes and a shortened time to progression, indicating that on-treatment changes in HPV16 ctDNA levels may predict responses to treatment with VB10.16 and atezolizumab in cervical cancer.

**Conclusions** Analysis of liquid biopsies in patients with HPV16-positive recurrent or metastatic cervical cancer, treated with VB10.16 in combination with atezolizumab indicate that monitoring HPV16 ctDNA may predict clinical outcome and duration of response in a HPV16-specific therapy setting.

**Trial Registration** NCT04405349

**Ethics Approval** The study was approved by the national regulatory authorities and Independent Ethic Committees (NCT04405349).