ANALYTICAL VALIDATION OF A NOVEL IMMUNOHISTOCHEMISTRY ASSAY TO DETERMINE NUCLEAR AHR EXPRESSION IN HUMAN BLADDER CANCER

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Background Aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that translocates to the nucleus upon ligand binding, and activates a target gene expression program, contributing to the immunosuppressive state of the tumor microenvironment.¹ IK-175 is a selective, oral, potent AHR antagonist that has demonstrated strong immune modulation, and robust tumor growth inhibition, either as a single agent or in combination with anti-PD-1, in mouse models. IK-175 is being evaluated in an ongoing phase 1 clinical study as a single agent and in combination with nivolumab for bladder cancer patients (NCT04200963). We hypothesized that nuclear AHR protein expression in tumors is an indicator of activated AHR signaling, and thus a potential predictive biomarker for the clinical response to IK-175. To test this hypothesis, we have developed a novel AHR immunohistochemistry (IHC) assay and analytically validated it in a Clinical Laboratory Improvement Amendments (CLIA) certified lab. We have successfully implemented it in our ongoing phase 1b clinical trial of IK-175 for prospective patient enrollment.

Methods The AHR IHC assays were developed in collaboration with Flagship Biosciences and Neogenomics on a Leica Bond RX and a CDx-compatible Leica Bond III platform, respectively. AHR expression pre-characterized cell lines, xenografts, and human bladder cancer samples were used to optimize the IHC conditions for specific and sensitive detection of nuclear AHR protein. The Neogenomics assay was further analytically validated for accuracy, sensitivity, specificity, and precision. The IHC assay developed in Flagship was used as an orthogonal comparator for accuracy testing. A cutoff value for AHR nuclear positivity was also determined by assessing n=200 human bladder cancer specimens.

Results To determine the prevalence of nuclear AHR expression in human bladder cancer, two hundred unique human bladder cancer specimens, including sixty bladder cancer blocks and 2 tumor microarrays, were analyzed. A cutoff of 65% tumor cells positive for 2+/3+ nuclear AHR was determined to enable a targeted enrollment of 1 in 3 to 5 patients screened. Assay accuracy, sensitivity, specificity, and precision were assessed, and reached 96%, 100%, 95%, and 100% respectively. The intra-pathologist concordance was also assessed to be 90% concordant. The assay passed the pre-defined acceptance criteria (>85%) in all parameters.

Conclusions A novel and robust nuclear AHR IHC assay for bladder cancer was developed and analytically validated in a CLIA certified lab and showed ≥95% accuracy, specificity, sensitivity, and precision, enabling its implementation in an IK-175 Ph1b clinical study (NCT04200963) for prospective patient enrichment.

Trial Registration NCT04200963

REFERENCE

1. Campesato L, Budhu S, Tchaicha J, et al. Blockade of the AHR restricts a Treg-macrophage suppressive axis induced by L-Kynurenine. *Nature Communications* 2020; **11**:4011

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