Background CD73 (ecto-5'-nucleotidase) is an ecto-nucleotidase that dephosphorylate AMP to form adenosine. Activation of adenosine signaling pathway in immune cells leads to the suppression of effector functions, down-regulate macrophage phagocytosis, inhibit pro-inflammatory cytokine release, as well as yield aberrantly differentiated dendritic cells producing pro-tumorigenic molecules.1 In the tumor microenvironment, adenosinergic negative feedback signaling facilitated immune suppression is considered an important mechanism for immune evasion of cancer cells.2,3 Combination of CD73 and anti-PD-1 antibody has shown promising activity in suppressing tumor growth. Hence, we developed AK119, an anti- human CD73 monoclonal antibody, and AK123,a bi-specific antibody targeting both PD-1 and CD73 for immune therapy of cancer.

Methods AK119 is a humanized antibody against CD73 and AK123 is a tetrameric bi-specific antibody targeting PD-1 and CD73. Binding assays of AK119 and AK123 to antigens, and antigen expressing cells were performed by using ELISA, Flowcytometry, and FACS assays. In-vitro assays to investigate the activity of AK119 and AK123 to inhibit CD73 enzymatic activity in modified CellTiter-Glo assay, to induce endocytosis of CD73, and to activate B cells were performed. Assay to evaluate AK123 activity on T cell activation were additionally performed. Moreover, the activities of AK119 and AK123 to mediate ADCC, CDC in CD73 expressing cells were also evaluated.

Results AK119 and AK123 could bind to its respective soluble or membrane antigens expressing on PBMCs, MDA-MB-231, and U87-MG cells with high affinity. Results from cell-based assays indicated that AK119 and AK123 effectively inhibited nucleotidase enzyme activity of CD73, mediated endocytosis of CD73, and induced B cell activation by upregulating CD69 and CD83 expression on B cells, and showed more robust CD73 blocking and B cell activation activities compared to leading clinical candidate targeting CD73. AK123 could also block PD-1/PD-L1 interaction and enhance T cell activation.

Conclusions In summary, AK119 and AK123 represent good preclinical biological properties, which support its further development as an anti-cancer immunotherapy or treating other diseases.

REFERENCES


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