COMPARISON OF FOUR IN VITRO CYTOTOXICITY ASSAYS FOR ASSESSING THE POTENCY OF CD3-BISPECIFIC ANTIBODIES REDIRECTING T CELLS TO KILL TUMOR TARGET CELLS

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Background Evaluation of cytotoxicity is a critical step in the selection of CD3-bispecific antibodies (BsAbs) for cancer immunotherapy. The objective of this study was to compare sensitivities, strengths, and limitations of four commonly used in vitro cytotoxicity assays to determine potency of CD3 BsAbs in a co-culture system composed of tumor target cells and human CD3+ T cells.

Methods

Lactate dehydrogenase (LDH) Release Assay: 786-O renal cancer cells were co-cultured with human CD3+ T cells in the presence of tool CD3 BsAbs or control antibodies. LDH release from membrane-damaged cells was measured using a fluorometric method (Promega). IncuCyte-based Assay: Co-culture plates as prepared for the LDH release assay were scanned by IncuCyte S3 system with 5 images captured per well; phase object confluence was measured. Killing Immune-Lysis Reaction (KILR) Assay: 786-O KILR cell pool generated by transduction of KILR retroparticles into the parental 786-O cells was co-cultured with human T cells. The release of tagged KILR reporter proteins from dying cells was detected by a luminescence method (Eurofins). Flow Cytometry–based Assay: U266 myeloma target cells were co-cultured with effector T cells. CD3 BsAbs-induced T-cell cytotoxicity, proliferation, and activation marker expression were evaluated using Intellitec iQue flow cytometer. Cytokine secretion from activated T cells in the co-culture systems was measured using Meso Scale Discovery assays.

Results All 4 in vitro cytotoxicity assays consistently demonstrated that tool CD3 BsAbs induced T cell-mediated tumor cytotoxicity in a dose-dependent and time-dependent manner. This was in agreement with other T-cell activation parameters in terms of INF-γ secretion, surface expression of CD25 and CD69, and T cell proliferation. The KILR assay was the most sensitive and specific assay. The fluorescence produced in LDH release assay was mainly contributed by the dead 786-O target cells with minimum contribution from the dead T cells. Automated imaging and phase object confluence analysis by IncuCyte selectively quantified tumor cell death in real-time without labeling the target cells. The flow cytometry–based assay was relatively time consuming, but allowed for measurement of multiple T-cell activation biomarkers simultaneously.

Conclusions All 4 in vitro cytotoxicity assays evaluated are suitable to assess the efficacy of CD3 BsAbs redirecting T-cell cytotoxicity against tumor cells. The LDH release assay, IncuCyte-based assay, and KILR assay may be more robust for screening of CD3 BsAbs, while flow cytometry–based assay is beneficial for confirmation of the lead clones.

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