Application Note

Cell Analysis

Tumor Cell Killing by T Cells

Quantifying the impact of a CD19-BiTE using real-time cell analysis, flow cytometry, and multiplex immunoassay

Authors

Lauren Jachimowicz, PhD,
Agilent Technologies, Inc. USA

Peifang Ye
Agilent, China

Garret Guenther, PhD
Agilent Technologies, Inc. USA

Kenneth Chan, PhD
Agilent Technologies, Inc. USA

Jeff Shurong Xue, PhD
Agilent Technologies, Inc. USA

Abstract

The ability to quantitatively correlate T cell biomarker expression or secretion with target cell killing is critical in tumor immunology studies. This application note demonstrates a workflow using the Agilent xCELLigence real-time cell analysis (RTCA) instrument with an Agilent NovoCyte flow cytometer to study both cytotoxic T lymphocyte (CTL)-mediated destruction of tumor cells and the corresponding secretion of cytokines and cytolytic proteins by CTLs. Bispecific T cell engagers (BiTEs) enhance the ability of CTLs to specifically recognize and eliminate tumors. This enhancement effect was evaluated using three distinct assays. Target cell death was monitored using an Agilent RTCA biosensor assay and a flow cytometry-based cytolysis assay, while secretion of cytokines and cytolytic proteins was quantified in a bead-based multiplex flow cytometry assay.

Introduction

Cancer immunotherapy is increasingly being evaluated as an approach to cancer treatment by harnessing the immune system to attack cancer cells. Both the adaptive and innate arms of the immune system play a pivotal role in a host's defense against tumors. CD8+ CTLs, a major component of the adaptive immune response, directly eliminate tumor cells by releasing cytolytic proteins such as granzymes, perforin, and granulysin, in addition to producing multiple cytokines.

The ability to correlate T cell biomarker expression/secretion with target cell killing is critical in both basic and applied studies of tumor immunology. Moving this research from the bench to the lab is critically important, but reliable tools are needed to translate *in vitro* protocols and results for use *in vivo*. This study uses a combination of biosensor-based technology and flow cytometry to evaluate both the target and effector cells in a T cell-mediated B cell killing assay. The Agilent xCELLigence RTCA biosensor technology provides a continuous readout of target cell viability, while flow cytometry allows simultaneous measurement of specific responses, such as target cell viability and effector cell function.

Representing a promising new class of therapeutics, BiTEs harness the power of the adaptive immune response by enhancing the ability of CTLs to specifically recognize and eliminate tumors. CD19-BiTEs are designed to bind CD3 on CTLs, as well as CD19 on cells of B cell lineage, simultaneously activating T cells and bringing them in close proximity to the B cells. This enhances the CTL effector function against various B cell-derived tumors. The capability of CD19-BiTEs to enhance the cytotoxic effects of T lymphocytes on a B-cancer cell line, Daudi cells, has been evaluated using three distinct assays (Figure 1). Target cell death was monitored using an RTCA biosensor assay and a flow cytometry-based cytolysis assay; while secretion of cytokines and cytolytic proteins were quantified in a bead-based multiplex flow cytometry assay to monitor the T cell response.

CD19-BiTE enhances T cell-mediated target cell killing in an xCELLigience RTCA biosensor assay

To demonstrate T cell-mediated target cell killing on the Agilent xCELLigence, human T cell killing of a B cell lymphoma cell line (Daudi cells) was assessed. Daudi cancer B cells were immobilized on an xCELLigence electronic microplate (Agilent E-Plate) that had been precoated with αCD40 antibody. Interaction between the cells and the gold micro-electrodes leads to increased electrical impedance, the magnitude of which correlates with cell number, size, and cell-substrate attachment quality. At 18 hours after the Daudi cells had been seeded, T cells enriched from primary PBMCs were added at an effector T cell to target Daudi cell ratio of 10:1. To measure if a BiTE can enhance T cell killing, CD19-BiTE or an αCD19 antibody control was also added. The impedance signal of the Daudi cell monolayer was recorded every 15 minutes, and is plotted using the unitless parameter Cell Index.

Uninterrupted growth and attachment of Daudi cells can be seen in wells with only Daudi cells (Figure 2, blue line), while no sustained impedance signal is generated from T cells alone (Figure 2, black line). Daudi cell growth is undisturbed by the addition of T cells and αCD19 antibody as a control (Figure 2, green line). However, a rapid decrease in Cell Index is observed with the addition of the CD19-BiTE, indicating that that Daudi cells are dying (Figure 2, orange line). These data demonstrate the capability of CD19-BiTE to enhance T cell-mediated B killing efficacy.

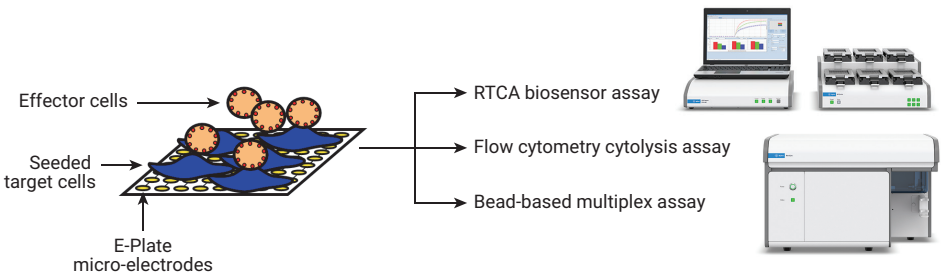


Figure 1. CD19-BiTEs enhance the cytotoxic effects of T lymphocytes on a B-cancer cell line, Daudi cells, using three distinct assays.

CD19-BiTE enhances T cell-mediated target cell killing in a flow cytometry-based cytolysis assay

The role of CD19-BiTE as a powerful facilitator of T cell-mediated killing of Daudi B cells was further confirmed using flow cytometry. Daudi cells were labeled with CFSE and cultured identically to the RTCA experiment described previously. At 48 hours after T cell addition, dead cells were detected with 7-AAD and acquired on the NovoCyte flow cytometer (Figures 3A and 3B). The presence of T cells and the αCD19 antibody did not significantly increase the frequency of Daudi cells (CFSE+) that were dead (7-AAD+) compared to the sample containing only Daudi cells. However, the addition of CD19-BiTE dramatically decreased the proportion of viable Daudi target cells; approximately 80% of Daudi cells were dead. These data correlate closely to the results obtained from RTCA, verifying that T cell-mediated B cell killing can be properly evaluated by flow cytometry or cellular impedance.

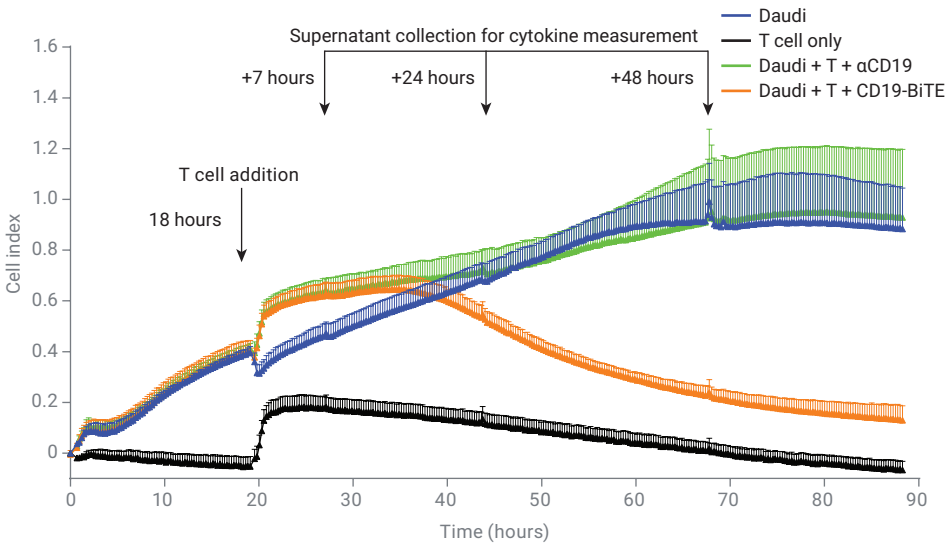


Figure 2. CD19-BiTE enhances T cell-mediated B cell killing measured by an RTCA biosensor assay. Daudi target cells were seeded at 50,000 cells/well in an Agilent 96-well E-Plate coated with αCD40 antibody. At 18 hours after Daudi cell seeding, enriched human effector T cells from primary PBMCs were added at a T:Daudi cells ratio of 10:1. Supernatant was collected at 7, 24, and 48 hours after the addition of T cells for protein measurement in the following experiment. Interaction of cells with gold micro-electrodes (biosensors) impedes the flow of electric current between electrodes. This impedance value is plotted as the unitless parameter Cell Index, and correlates with cell number, size, and cell-substrate attachment quality. The impedance signal was recorded every 15 minutes.

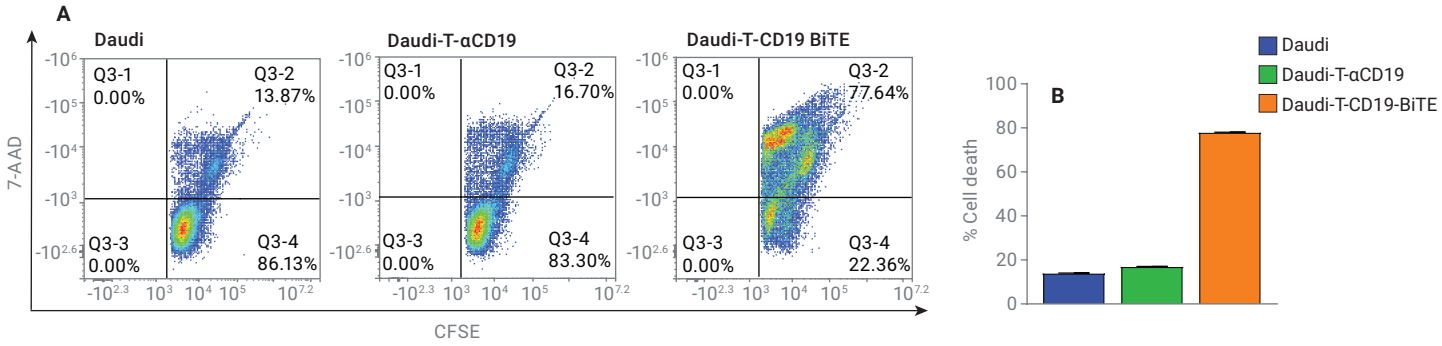
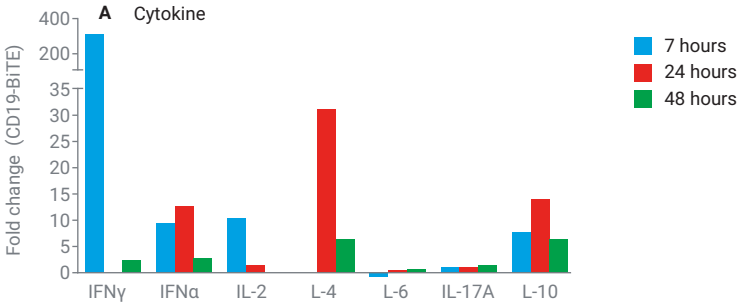


Figure 3. CD19-BiTE enhances T cell-mediated B cell killing measured by flow cytometry staining. Daudi cells were labeled with CFSE, and seeded at 100,000 cells/well. At 18 hours after Daudi cell seeding, enriched T cells were added with CD19-BiTE or an anti-CD19 antibody. At 48 hours after T cell addition, 7-AAD was added to identify dead cells by flow cytometry. A) Represents FCM plots. B) Frequency of CFSE+ 7-AAD+ dead Daudi cells.

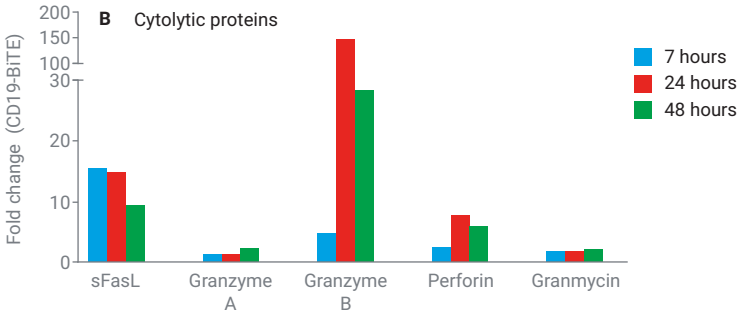
CD19-BiTE enhances cytokine and cytolytic protein secretion by T cells in a bead-based immunoassay

To further study the effects of CD19-BiTE on T cell activation and function, cytokine and cytolytic protein secretion were measured. Cells were cultured as described in Figure 2, and a supernatant was taken 7, 24, and 48 hours after the addition of T cells. This was done to measure 13 human proteins known to affect T cell function with a bead-based multiplex assay (LEGENDplex Human CD8/NK panel, Biolegend) (Figure 4). Consistent with our findings from RTCA and flow-based cytolysis assays, an increased expression of CTL-associated proteins was observed. These data demonstrate that the presence of CD19-BiTE significantly enhances production of cytokines and effector molecules that mediate and sustain target cell killing.

At 7 hours after the addition of effector T cells, cytokines associated with CTL response, such as IFNγ, TNFα, and IL-2, are increased 300-, 9-, and 10-fold, respectively. Secretion of cytolytic proteins, such as FasL, Granzyme B, and perforin, are also dramatically increased by 24 hours, consistent with the CTL killing response seen. These data demonstrate that CD19-BiTE enhances T cell-mediated B cell killing by increasing the production of cytokines and cytolytic proteins essential for a robust CTL response.



7 hours	310.6	9.3	10.4	N/A	0.3	1.2	7.7
24 hours	N/A	12.7	1.3	31.2	0.5	1.2	13.9
48 hours	2.2	2.9	0.0	6.5	0.4	1.7	6.2



7 hours	15.6	1.1	4.8	2.4	1.6
24 hours	14.5	1.2	143.6	7.7	1.6
48 hours	9.2	2.5	28.2	6.0	1.9

Figure 4. CD19-BiTE enhances the cytotoxic activity of T cells in a bead-based multiplex immunoassay. Daudi target cells were seeded at 50,000 cells/well in an Agilent 96-well E-Plate coated with αCD40 antibody. At 18 hours after Daudi cell seeding, enriched human effector T cells from primary PBMCs were added at a T:Daudi cells ratio of 10:1. Supernatant was collected at 7, 24, and 48 hours after the addition of T cells for bead-based multiplex immunoassay (LEGENDplex Human CD8/NK Panel). The relative fold change between protein expression of Daudi + T + CD19BiTE to Daudi + T + αCD19 was determined for cytokines (A) and cytolytic proteins (B).

Conclusion

We have coupled quantitative cell killing assays with biomarker quantitation to provide an in-depth view of how CD19-BiTE affects T-mediated killing of B cells in a single workflow. The continuous monitoring of cell number, size, and attachment quality using RTCA enables the quantitative and kinetic assessment of the killing process. This is simultaneously corroborated by flow cytometry. Linking this cell killing data with quantitative analysis of cytokine and effector protein production allows simultaneous analysis of T cell activation and function. This workflow, which integrates both cellular and molecular phenomena, advances current methods of analysis for both basic and applied research of cancer immunotherapy.

www.agilent.com/chem

Printed in the USA, November 1, 2019
5994-1022EN
AN 14
DE.3863541667

Agilent
Trusted Answers