POSTER # 14

Redirected Lysis Assay as an Efficient Potency Assay to Assess TILs for Immunotherapy

Aishwarya Gokuldass, Michelle A Blaskovich, Isabella Kopits, Brian Rabinovich, Michael T Lotze

Lion Biotechnologies, Inc. 999 Skyway Road, Suite 150, San Carlos, CA 94070

Abstract

Adoptive T-cell therapy (ACT) with tumor infiltrating lymphocytes (TILs) is a promising therapy for patients with melanoma and other cancers. TILs involve culturing tumor fragments with IL-2 for 11-21 days, and re-infusing them into the patient following a rapid expansion protocol involving mitogenic anti-CD3 and irradiated PBMC. T-cells cultured from the tumor fragments are a heterogeneous mixture of cells representing CD4+, CD8+, and NK-cells with varying cytolytic activity and specificities. We developed a surrogate target cell line to evaluate the lytic potential of TILs in a Bioluminescent Redirected Lysis Assay (BRLA), enabling assessment of T-cell mediated killing in the absence of autologous tumor cells. Cytolytic activity can be assessed with and without engaging the T-cell receptor in 1-4 hours, assessing T-cell killing engaging the T-cell receptor and without, so-called lymphokine activated killer activities (LAK).

Methods

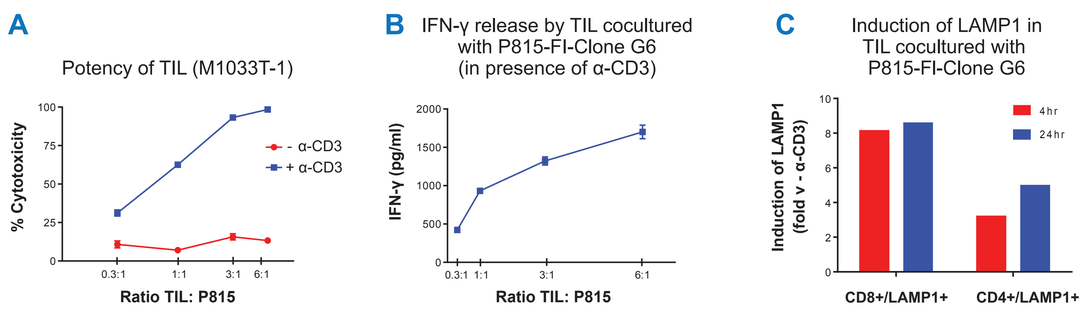
- Mouse mastocytoma P815 cells expressing the endogenous CD16 Fc receptor can bind anti-CD3ε (OKT3), providing a potent TCR activation signal as a target cell line.
- The P815 Clone G6 was transduced with a lentiviral vector based on eGFP and Firefly Luciferase, sorted and cloned using the BD FACSAria II. Clone G6 was selected based on eGFP intensity analyzed using Intellicyte iQue Screener.
- Target cells and TILs of interest were cocultured +/- OKT3 to assess TCR activation (specific killing) or non-specific (lymphokine activated killing, LAK) respectively.
- Following 4hr of incubation, Luciferin was added to the wells and incubated for 5 min. Bioluminescence intensity was read using a luminometer. Percent cytotoxicity and survival were calculated using the following formula:

% Survival = (experimental survival - minimum)/(maximum signal - minimum signal) x 100;
% Cytotoxicity = 100 - (% Survival)

- Interferon gamma (IFN-γ) release in the media supernatant of cocultured TILs was analyzed by ELISA, and LAMP1 (CD107a, clone eBioH4A3) expression on TILs was analyzed on a flow cytometer to evaluate the cytotoxic potency of TILs.

Results

Figure 1. BRLA: cytotoxic efficiency of TILs correlates with IFN-γ and LAMP1



Assay for TILs. A) Bioluminescent Redirected Lysis Assay: Percentage Cytotoxicity of TIL M1033T-1 when cocultured with P815 Clone G6 (with and without anti-CD3) at individual Effector: Target ratios. B) ELISA data showing amount of IFN-γ released against different ratios of effector to target cells. C) LAMP1 (%) expressed by TIL M1033T-1 when cocultured with P815 Clone G6 in the presence of anti-CD3 at a ratio of 1:1 effector to target cells for 4hr and 24hr coculture.

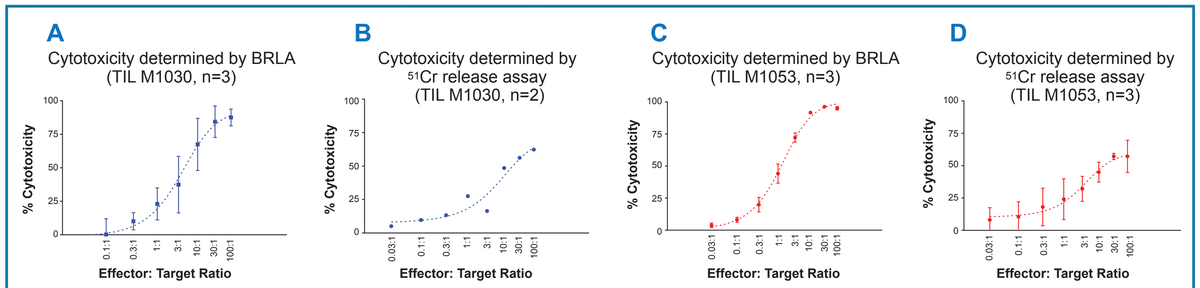
Disclosure and Funding Statement

This study was funded by Lion Biotechnologies, Inc. AG, MAB, IK, BR, and MTL are employees of Lion Biotechnologies, Inc. and have stock options.

Acknowledgment

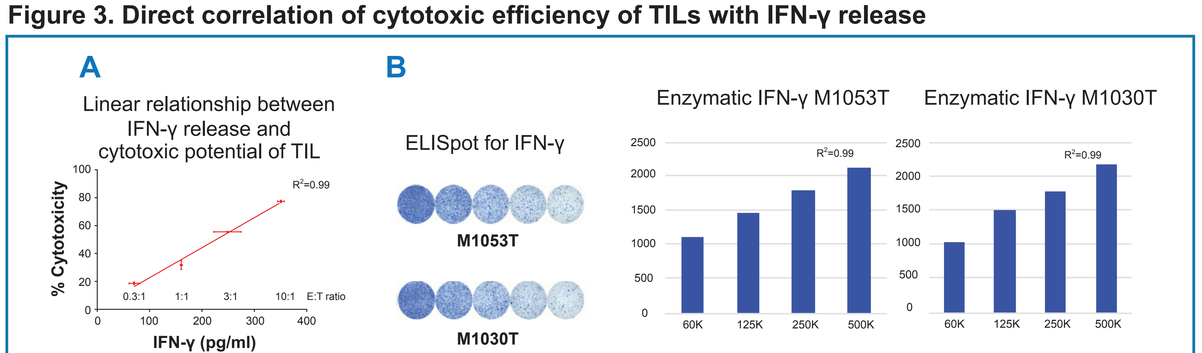
All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. Graphic services were provided by AOIC, LLC, and were funded by Lion Biotechnologies, Inc.

Figure 2. BRLA has comparable dynamic range compared with the standard chromium release assay



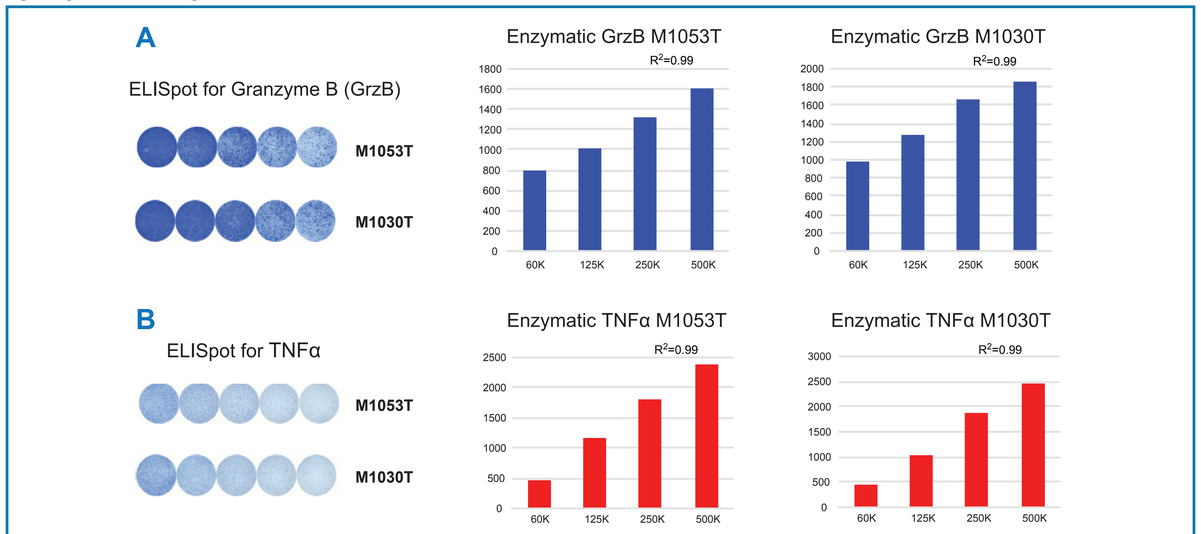
Three Log Dynamic Range for BRL Assay. A,B) Comparison of redirected lysis assay with standard chromium release assay for TIL M1030. Cytotoxicity measured as $LU_{50}/1x10^6$ TIL by BRLA is 26±16 and by chromium release assay is 22. C,D) Comparison of Redirected lysis assay with standard chromium release assay for TIL M1053 also showing lytic unit of the TIL by BRLA as 70±17 and chromium assay as 14±5. Comparison of two assay represents/shows comparable reliability of BRLA to chromium release assay.

Figure 3. Direct correlation of cytotoxic efficiency of TILs with IFN-γ release



A) Graphical representation of IFN-γ release and cytotoxic potential of M1053T showing a linear relationship. B) ELISpot data demonstrating IFN-γ release by the M1053T and M1030T TIL cells.

Figure 4. Direct correlation of Granzyme B and TNFα measures of M1053T and M1030T with cytolytic activity in BRLA



A) ELISpot data showing Granzyme B release by M1053T and M1030T. B) ELISpot measures showing TNFα release by M1053T and M1030T. This data confirms the potency of the TILs shown by the BRLA.

Conclusions

- The Bioluminescent Redirected Lysis Assay (BRLA) requires no radionuclides and is as efficient and sensitive as traditional cytotoxicity assays.
- Flow cytometric assessment of LAMP1 expression on TILs at individual time points demonstrates degranulation of cytotoxic T-cells relative to the potency shown by BRLA.
- The BRLA demonstrates similar to better potency than standard chromium release assay.
- BRLA also enables evaluation of the potency of TIL lytic activity. Comparison of BRLA with chromium release assay shows the efficiency and reliability of BRLA.
- BRLA has a linear relationship with IFN-γ release by TILs.
- Release assay of IFN-γ, TNFα, and Granzyme B by ELISpot is consistent with the cytotoxic efficiency of the TILs evaluated by BRLA.
- Future efforts will assess and contrast IFN-γ release and cytotoxicity assays as potency/release assays for TILs.

Presented at Society for Immunotherapy of Cancer in National Harbor, Maryland, USA, November 9-13, 2016