

INTRODUCTION

The Tumor Microenvironment (TME) has a key role in Solid Tumor Therapy Screening. We have developed a 3D *ex vivo* immunosuppressive assay mimicking the TME. It enables both allogenic & autologous tumor lysis by expanded Tumor Infiltrate Lymphocytes (TILs). It is a valuable 3D assay to study the activity of immune therapy drugs in patient sample.

OBJECTIVE

We aim to develop a robust *in vitro* platform to speed-up Immune Oncology Drug Screening able to reliably predict patient responses to Immune Therapy.

MATERIAL & METHODS

TME-aligned immunosuppressor media was produced by conditioned media from activated human Mesenchymal Stem Cells (hMSC). The TILs were expanded from patient-derived tumor samples and used for tumor killing potential evaluation. Target tumor cells were obtained from different sources: a) Isolated from patient-derived material and frozen until use in experiments with autologous or allogenic TILs or b) Tumor cell lines purchased from ATCC. The cells were mixed according to desired Effector-Target (E:T) ratios and embedded in 3D matrix in presence of TME-aligned media and immune therapy compounds, as Immune Checkpoint inhibitors (ImmChPi). The cell retrieval was performed at the end of desired timepoints and tumor cell killing and TILs activation profile were analysed by flow cytometry. Informed consent for study participation and approval by the ethical committees were obtained.

RESULTS

Schematic View of 3D IO TME-Aligned Assay: Tumor Cell Killing by TILs/T-Cells

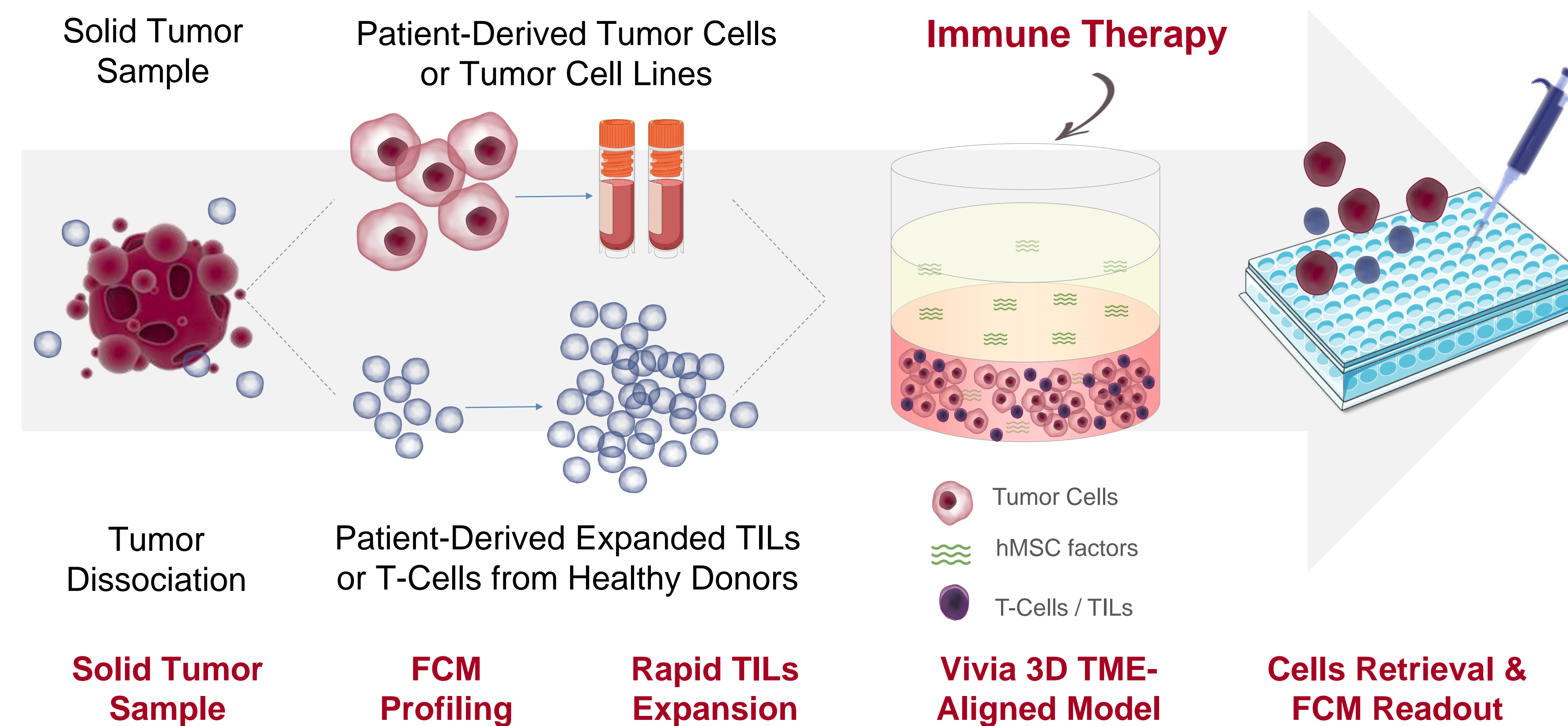


Figure 1. The flexible 3D IO TME-Aligned Assay can be performed using Tumor Cell lines or Patient Tumor Cells, the effector Cells can be Healthy T-cells or Autologous TILs. The exclusive Activated hMSC derived media combined with an Inflammatory TME provides naïve-like conditions as Acidic pH & Low glucose, mimicking native TME. Final endpoint cells retrieval allow full Mode of Action description.

Ovarian Tumor Killing by Healthy T-Cells Enhanced by IO Drugs

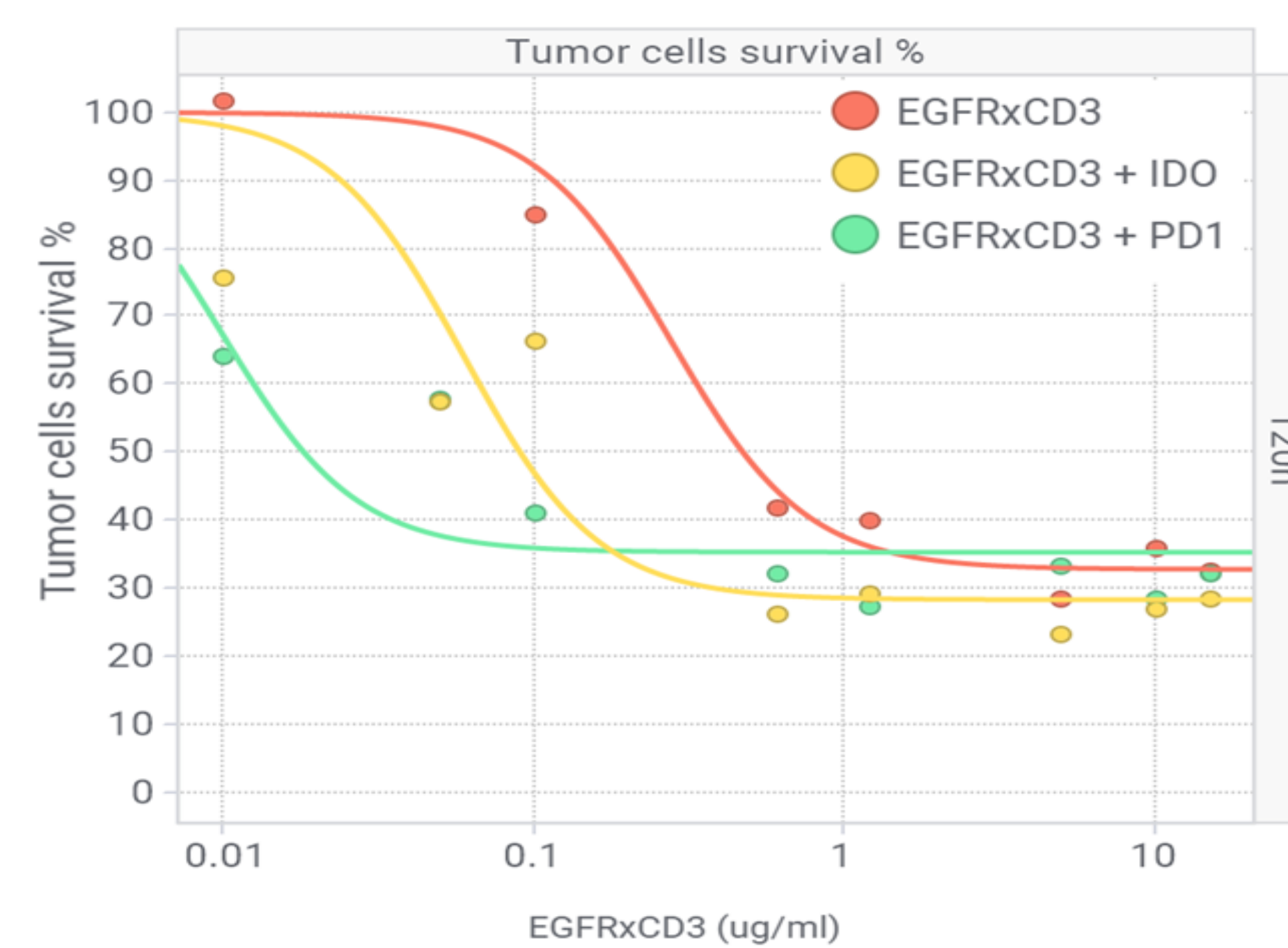


Figure 5. Patient-derived Ovarian Tumor Cells Killing by allogenic healthy T-Cells was enhanced in presence of ImmChPi IDO inhibitor and anti-PD-1 antibody in presence of BsAb Therapy.

Autologous TILs Kill 40% Ovarian Cancer Cells in 24h Incubation

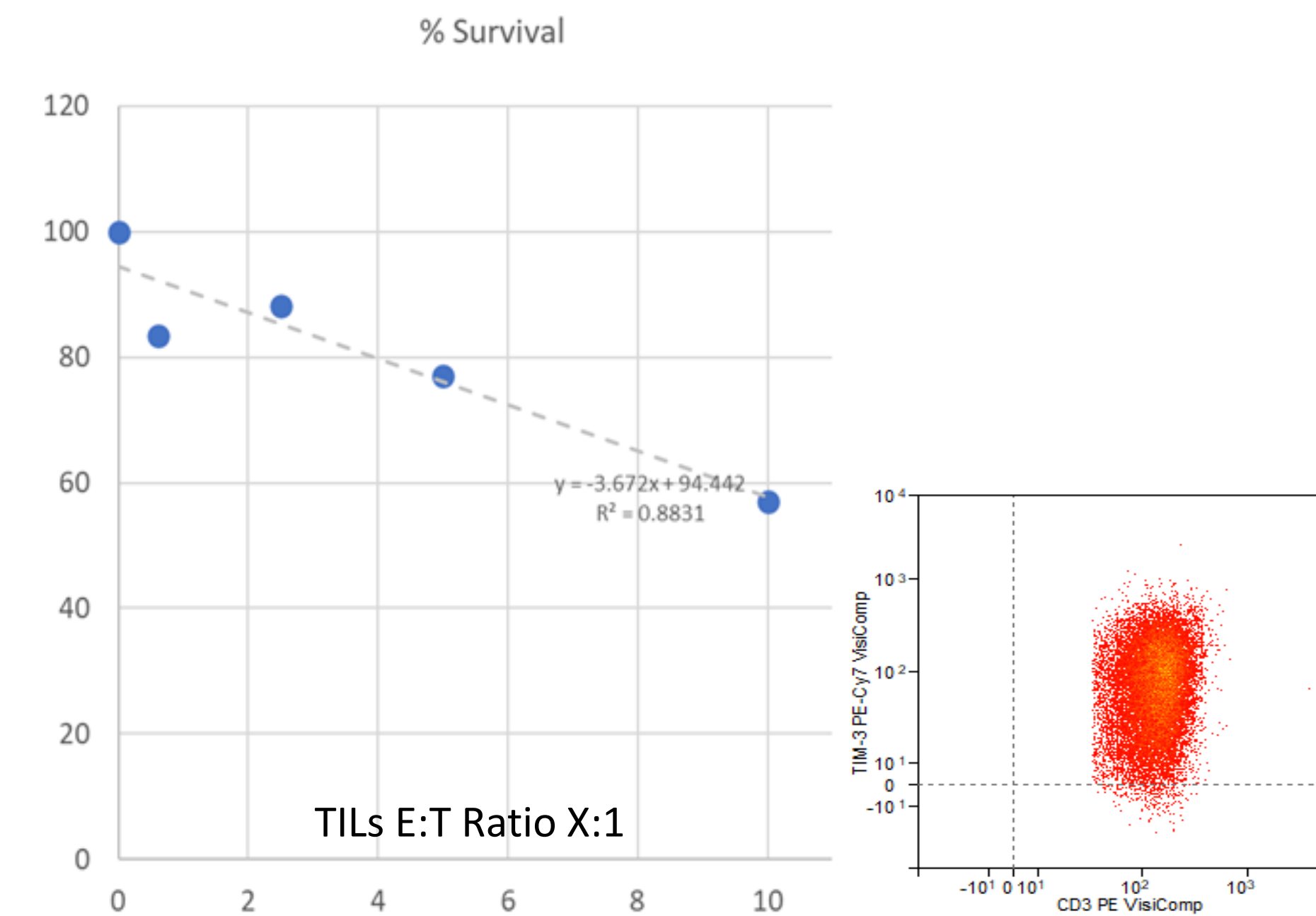


Figure 6. Left: Expanded TILs were able to kill 40% of autologous patient-derived ovarian tumor cells in 24h in TME-Aligned Model. Right: about 75% from patient-derived TILs express the ImmChp TIM3.

TME-Aligned Mimics Immunosuppressed TME

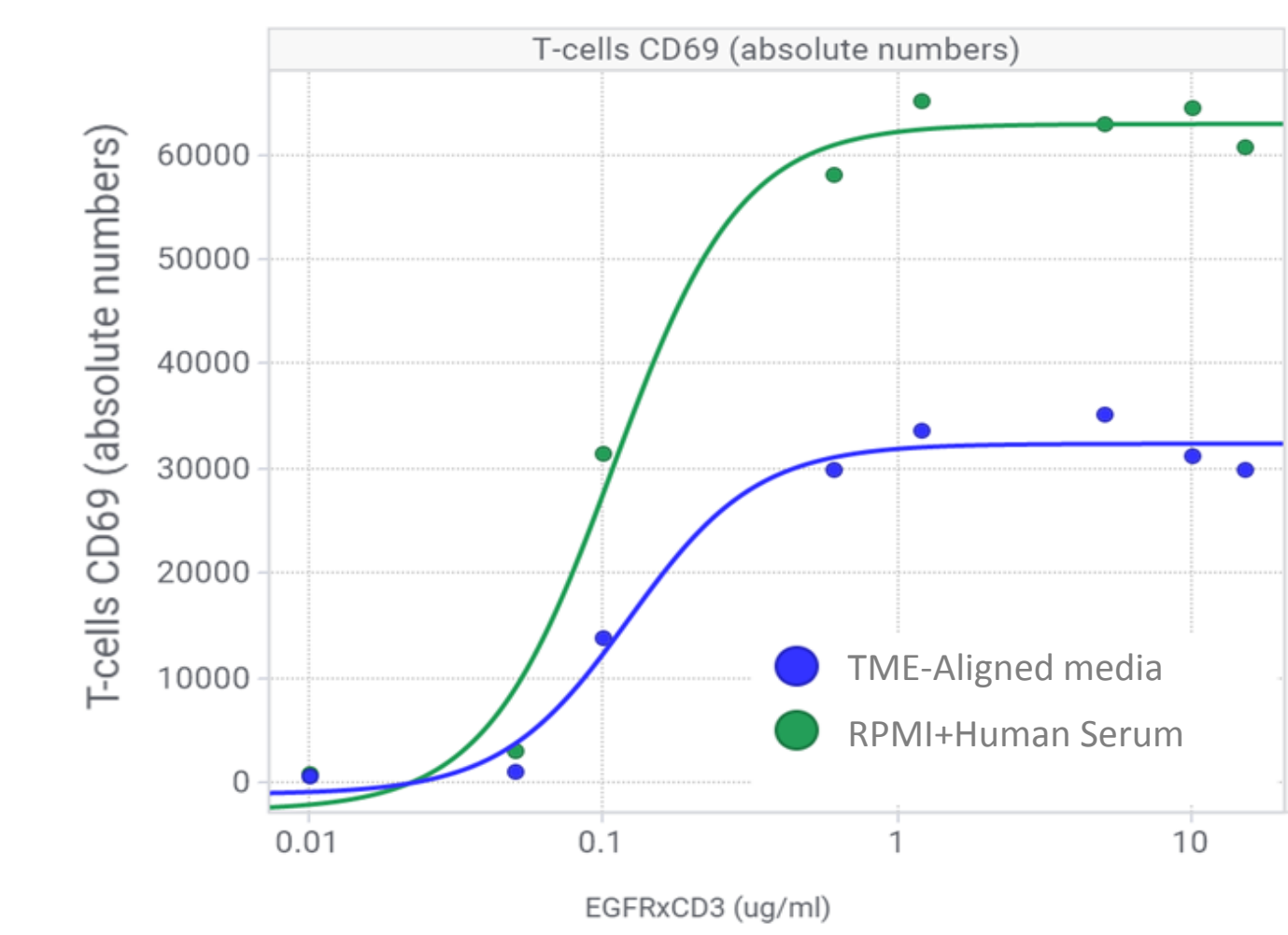


Figure 2. T-Cells activation marker CD69 expression is partially inhibited in TME-Aligned model in presence of bispecific antibodies (BsAb), compared with conventional culture methods.

Anti-PD-1 Enhances Healthy T-Cell Activation & Tumor Killing

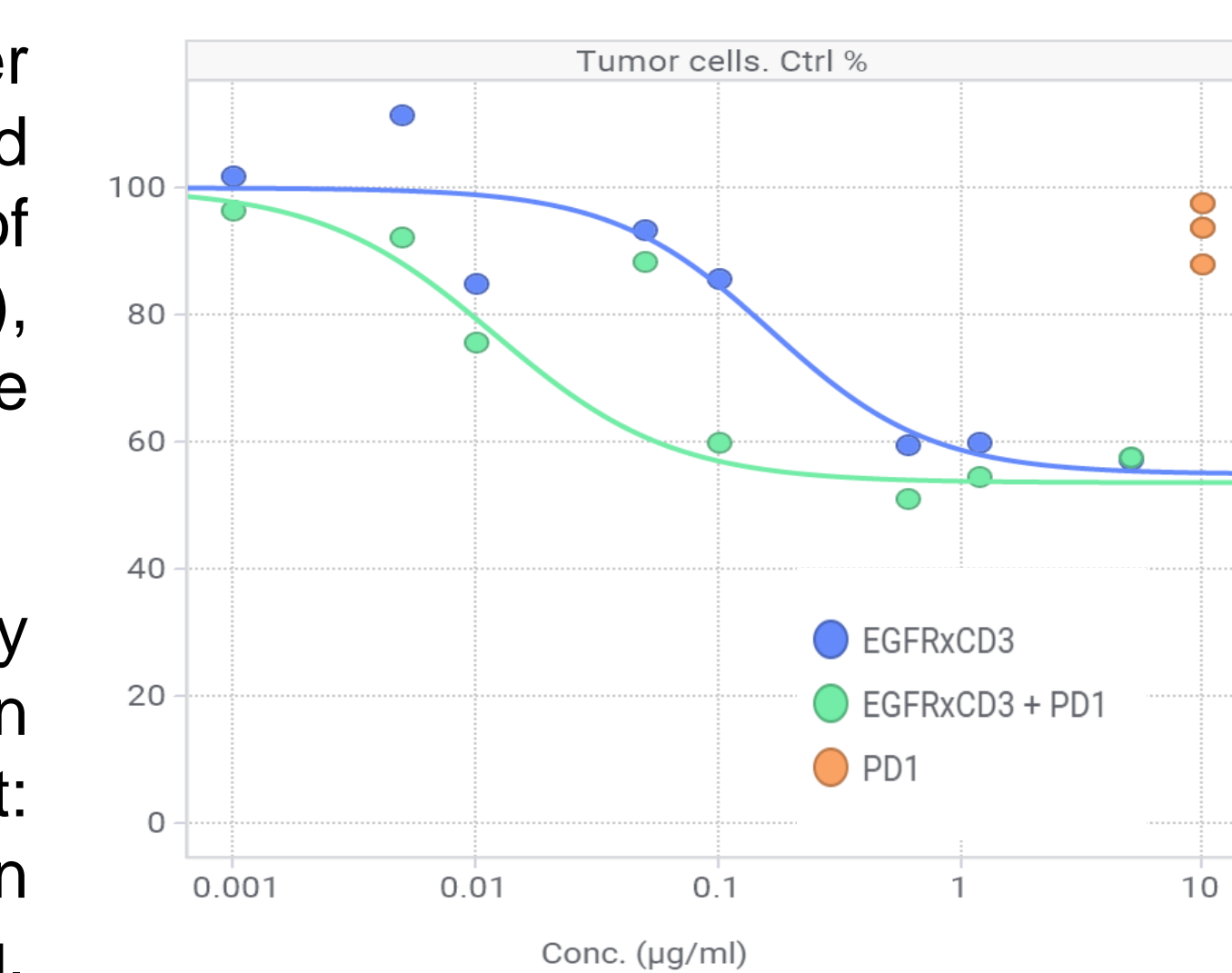
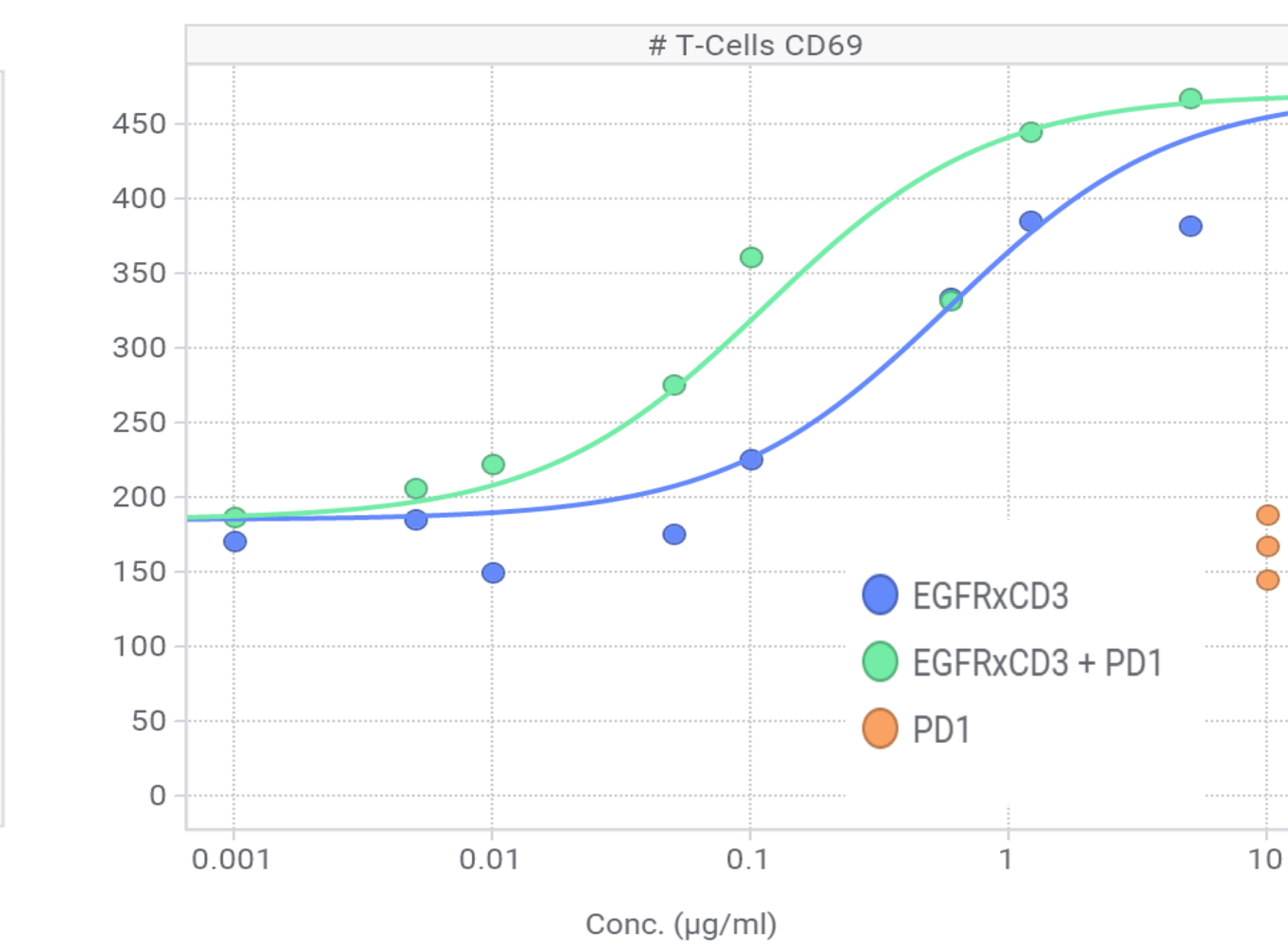


Figure 3. Up right: Anti-PD-1 antibody enhances healthy T-Cells activation driven by BsAb therapy. Bottom right: T-Cells activation reflects on increased allogenic tumor cells Killing.

Adding TIM3 Antibody Enhances Killing of Tumor Cells in a Dose Dependent Manner

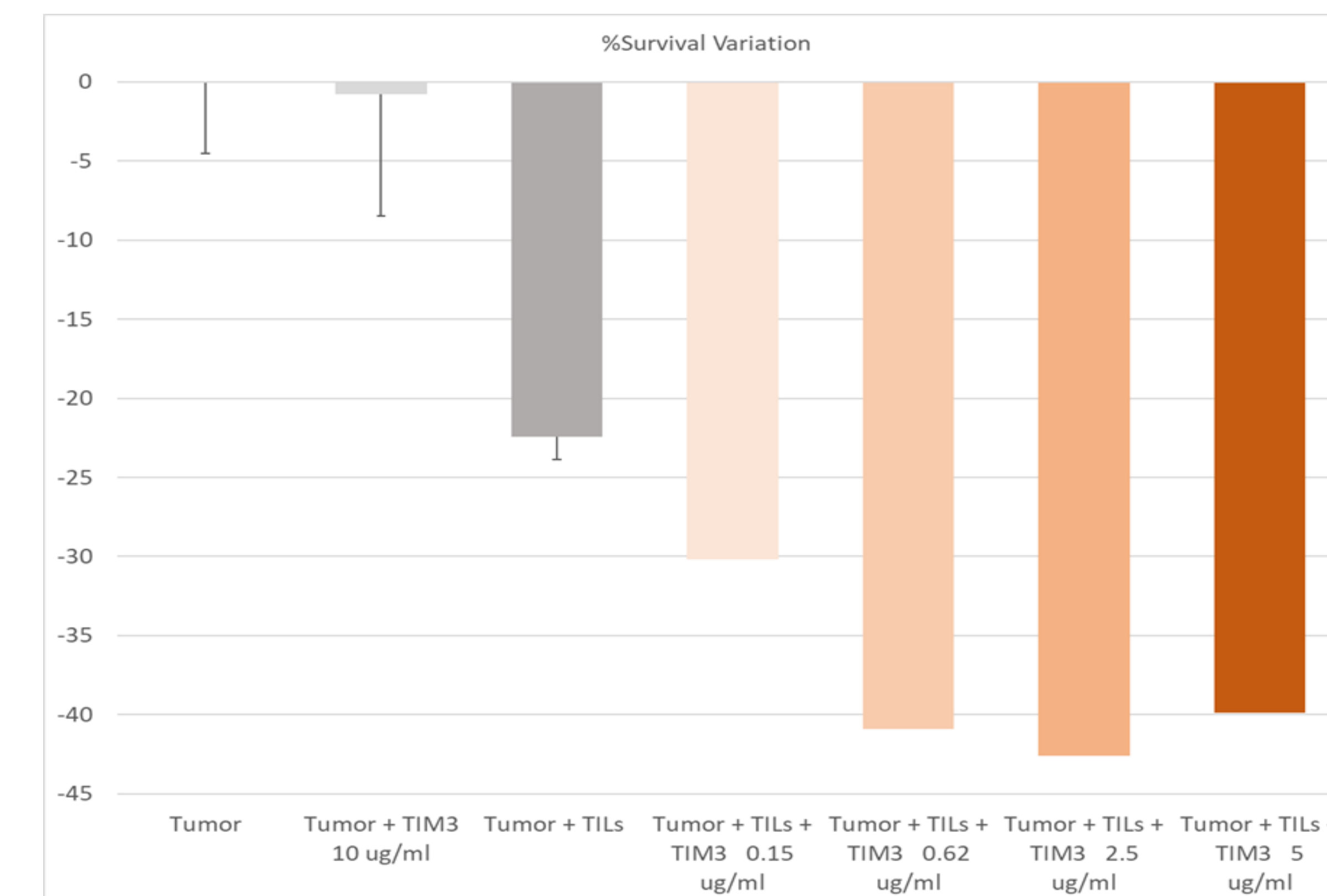


Figure 7. ImmChPi anti-TIM3 IO antibody enhanced tumor cell killing by autologous TILs in TME-Aligned Model.

Ovarian Tumor Killing by Healthy T-Cells Driven by BsAb

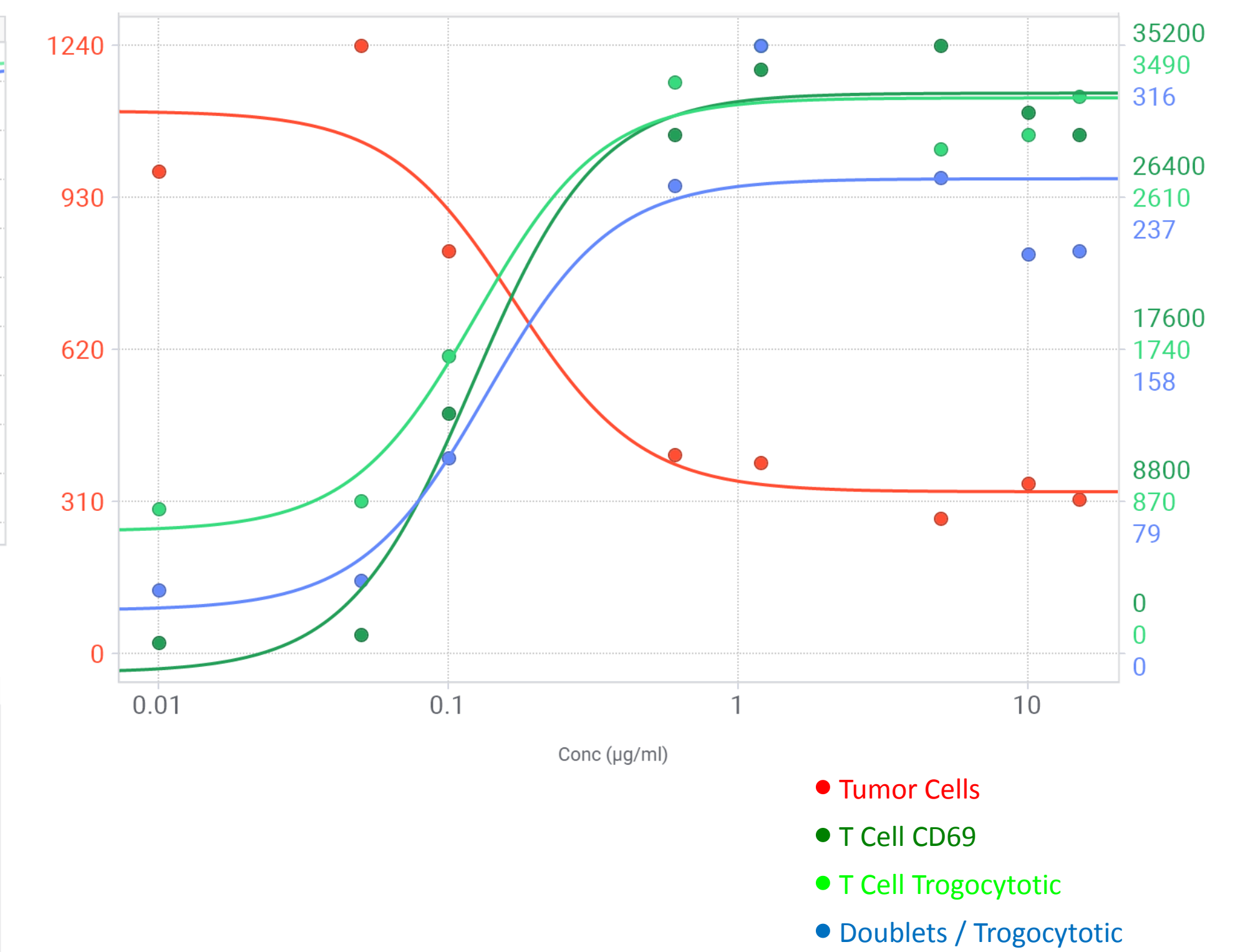


Figure 4. Upon BsAbs therapy, an increased patient-derived ovarian tumor killing (Red, left Y-scale) healthy T-Cells activation (Dark Green, Right Y-scale), Trogocytic T-Cells detection (Light Green, Right Y-scale) and doublets Tumor-T-Cells population (Blue, Right Y-scale) is observed.

CONCLUSIONS

The Novel TME-Aligned 3D IO Assay is a reliable, and powerful tool to study the mode of action of tumor cells lysis by healthy T-Cells or expanded TILs. Immune Therapy Drugs Screening can be performed in autologous or allogenic models and using different E:T ratios, allowing full mode of action description of Bi or Multispecific antibodies, ImmChPi and others, and opens a new door for therapy prediction studies in patient's material.

CONTACT INFORMATION

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