

Introduction

Solid tumors have a complex tumor microenvironment with deregulated extracellular matrix (ECM) that can modulate both Chimeric Antigen Receptor (CAR) T cell function and tumor cell survival.

There is a need for in vitro assays that can evaluate the impact of ECM on CAR T cell mediated tumor cell cytolysis. This study presents the utility of a novel invasion assay for CAR T cells that measures cytolysis of tumor cells in Matrigel in real-time using both label-free impedance and fluorescence-based imaging.

Aberrant fibroblast growth factor (FGF) receptor (FGFR) signaling is widely detected in 5 to 10% of all carcinomas.¹ FGFs can be bound by constituents of the extracellular matrices and can be accessed by tumors cells for survival and proliferation.² This study tested the inhibition of FGF signaling in combination with CAR T cells to target squamous cell carcinoma (SCC) cells in the presence of an ECM that acts as reservoir of FGF and presents a challenge for lymphocyte invasion.

Experimental materials and methods

Cell culture: A431, MCF-7, and U2OS cells were transduced with eLenti Red and eLenti Green (Agilent Technologies) to generate target cells stably expressing nuclear-localized mKate2 red fluorescent protein (RFP) or green fluorescent protein (GFP) .

ECM invasion and cytotoxicity assay: A431-red (7000 cells), U2OS-red (7000 cells) and MCF7-red (25000 cells) were seeded per well in E-Plate VIEW 96 plates. After approximately 24 hours (h), DMEM was aspirated and Matrigel supplemented with 10% FBS (6 mg/mL, 50 μ L/well) was added over the cells. Epithelial cell adhesion molecule (EpCAM) CAR T cells were seeded over the Matrigel layer after 2 h.

Impedance and live-cell imaging data collected on the Agilent xCELLigence Real-Time Cell Analysis (RTCA) eSight was used to determine tumor cell growth. The loss of impedance and fluorescence signal served as markers of cytolysis. Pemigatinib (5 μ M and 10 μ M) was used to evaluate the role of FGFR signaling. Percent cytolysis was calculated from normalized cell impedance and imaging (red fluorescence) data on the xCELLigence RTCA eSight software.

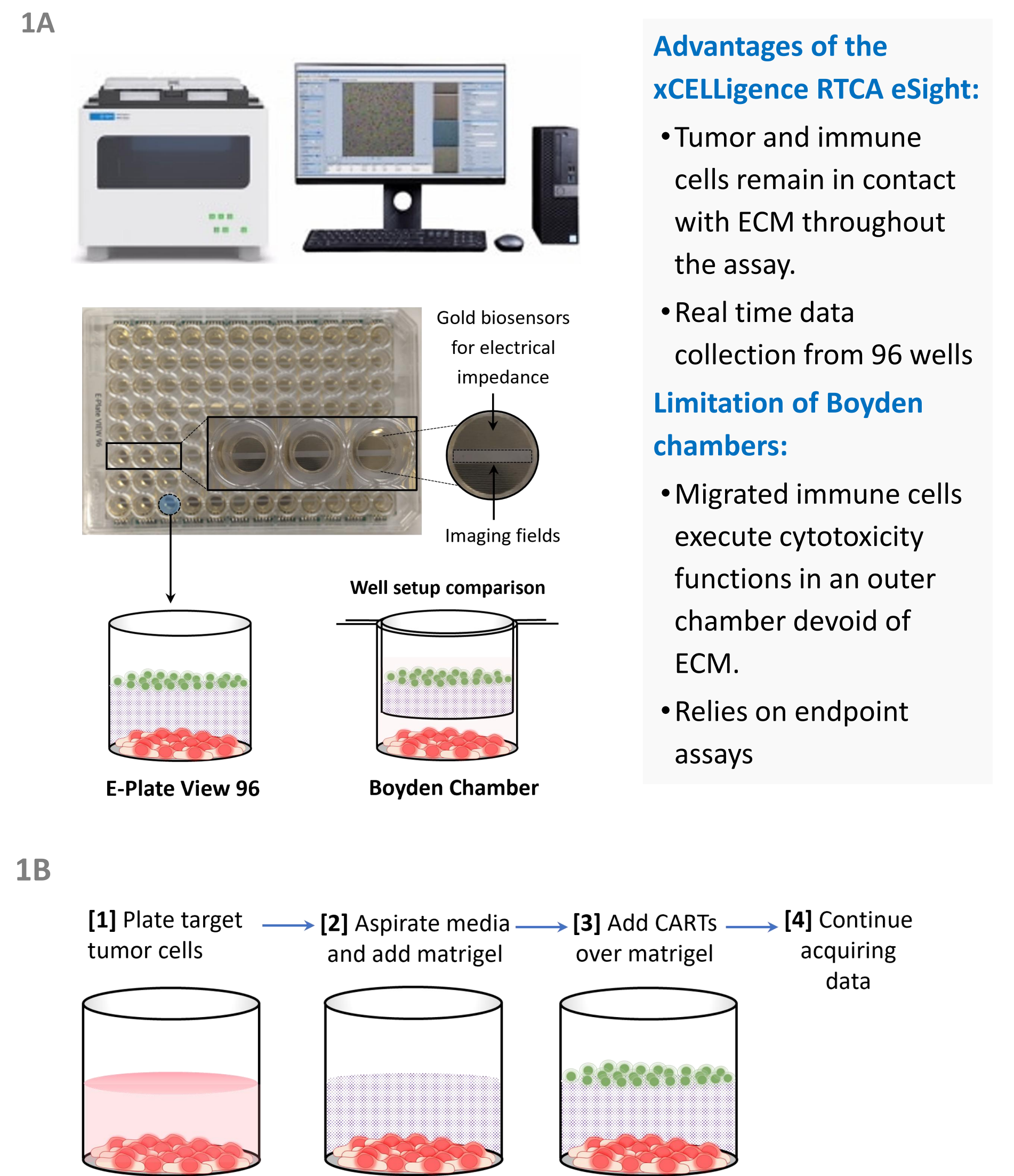
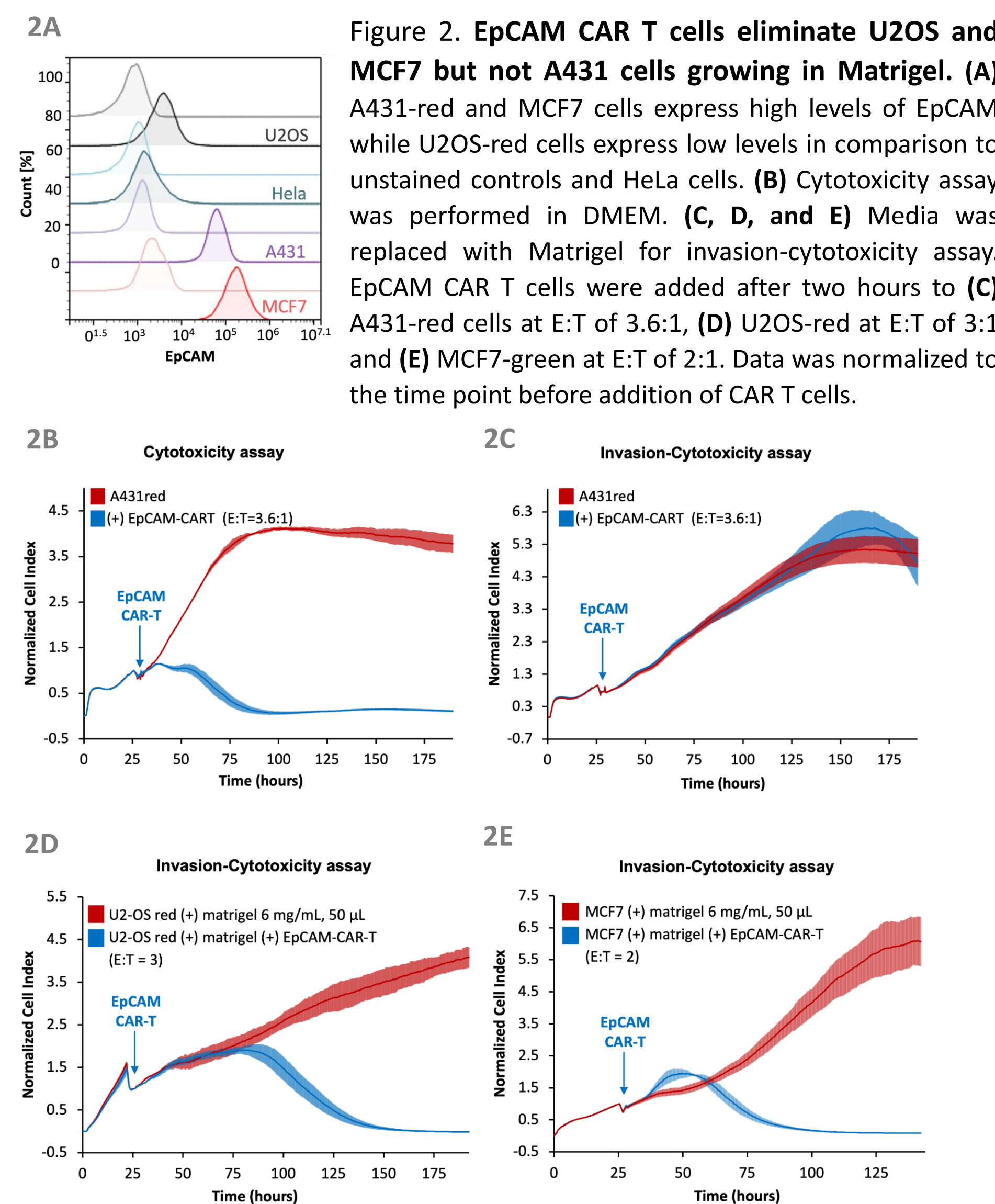


Figure 1. **Schematic representation of the setup for invasion and cytotoxicity assays.** (1A) Agilent xCELLigence RTCA eSight instrument E-Plate VIEW 96 and well setup (1B) Experiment outline CAR T cells (green) were added after the polymerization of Matrigel over the target A431 cells (red).

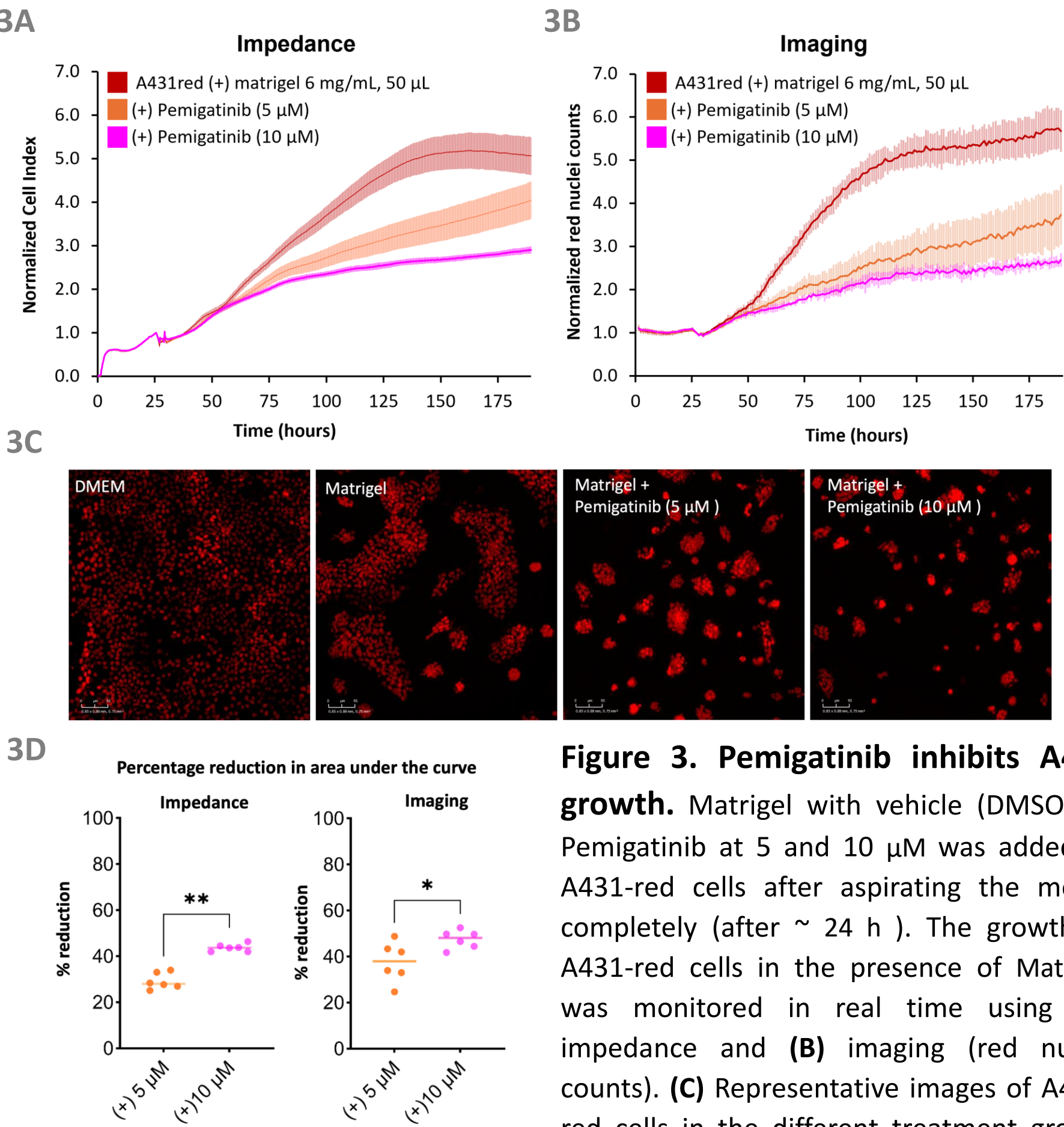
Results and Discussion

Cytotoxicity of EpCAM CAR T cells against U2OS and MCF7 and A431 cells



Results and Discussion

Evaluating Pemigatinib for inhibiting the growth of A431 cells in Matrigel



Evaluating Pemigatinib and EpCAM CAR T combination for A431 cells

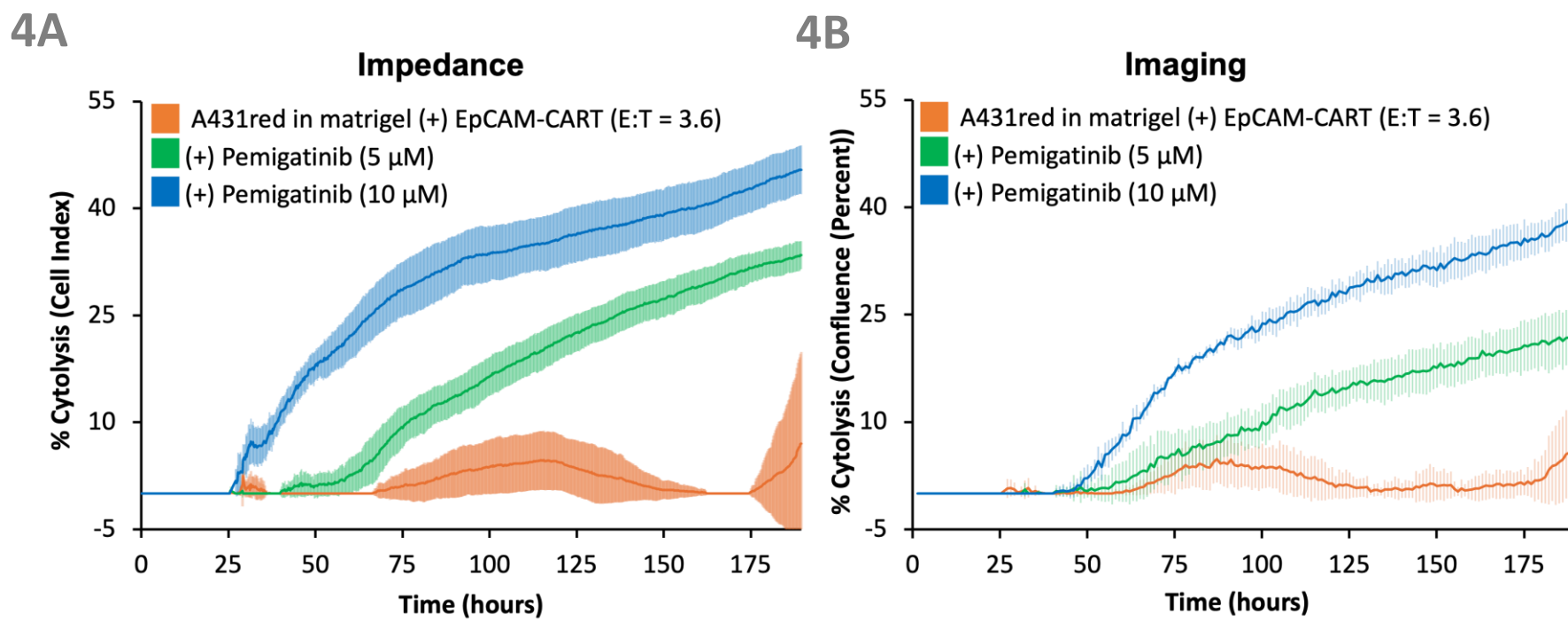


Figure 4. **EpCAM CAR T mediated killing of A431 cells in invasion-cytotoxicity assays is enhanced in presence of Pemigatinib.** (A) Percent cytolysis was computed from (A) Normalized Cell Index (NCI) and (B) confluence percentage in the red fluorescence channel computed from live cell imaging data.

A431 response to Pemigatinib and increased dose of EpCAM CAR T cells

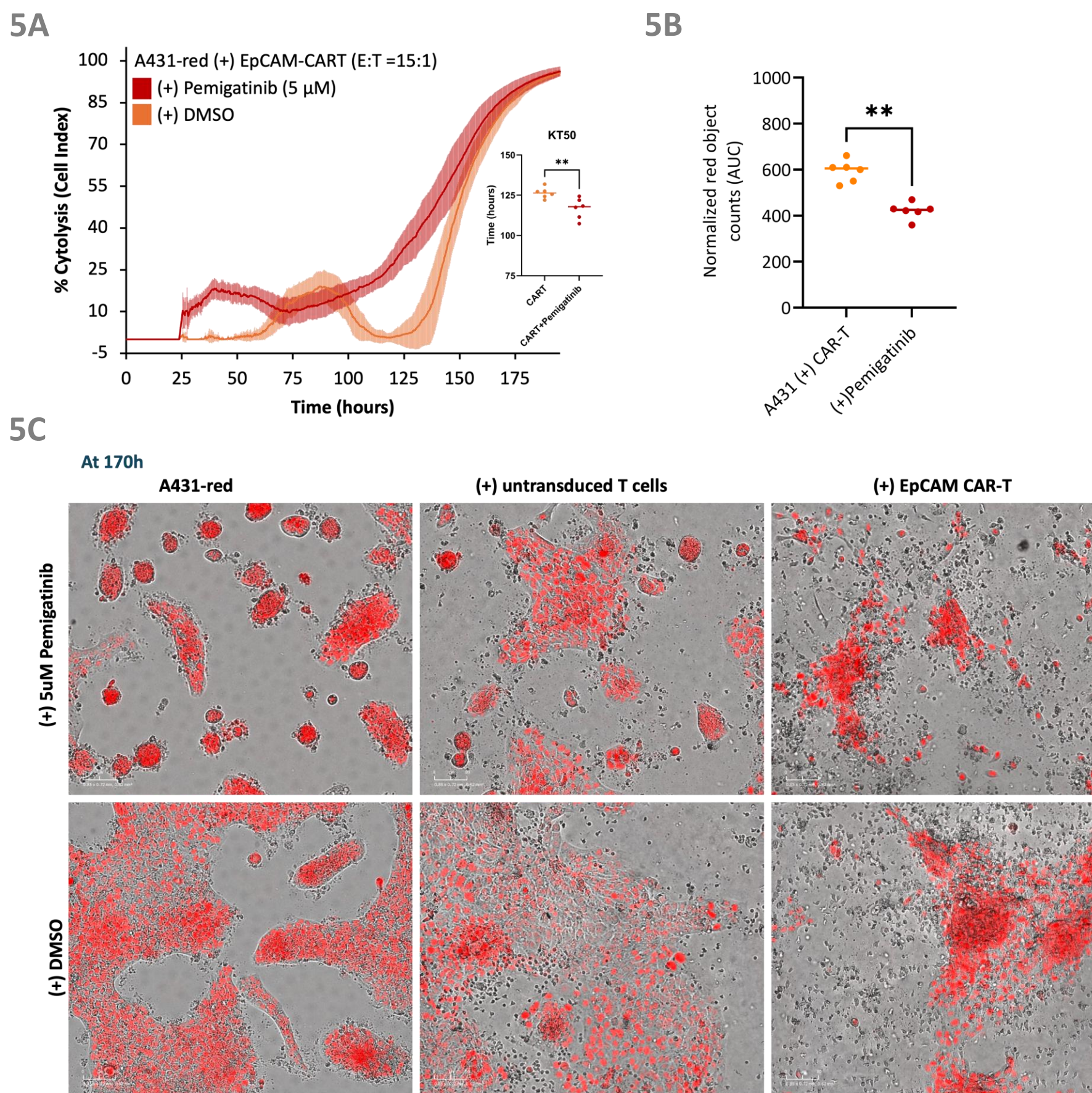


Figure 5. **Pemigatinib reduces A431 growth in the presence of Matrigel to enhance tumor cytolysis by EpCAM CAR T cells at E:T of 15:1.** (A) Percent cytolysis achieved by EpCAM CAR T cells (E:T = 15:1) in the presence and absence of Pemigatinib treatment was calculated separately with control T cells for each condition as reference and combined in Microsoft Excel. Time to achieve 50% killing (KT50) was compared using Mann-Whitney test in Prism (B) Area under the curve from normalized red nuclei counts in each well calculated on the xCELLigence RTCA eSight software showed significantly efficient control of tumor growth by EpCAM CAR T cells in the presence of Pemigatinib. Mann-Whitney T-test was performed on GraphPad Prism. **p=0.0022. (C) Representative images of A431-red cells taken at 170 hours shows that although untransduced and EpCAM CAR T cells (unlabeled) can invade the Matrigel, effective reduction in red nuclei count is achieved only by CAR T cells.

Conclusions

- EpCAM CAR T cells eliminate A431 carcinoma cells in simple cytotoxicity assays but fail to control the growth in invasion assays in the presence of Matrigel.
- The reduction in growth of A431 achieved with Pemigatinib enhanced the control of tumor growth by the EpCAM CAR T cells.
- This assay demonstrates that label-free impedance and fluorescence imaging on the Agilent xCELLigence RTCA eSight can be used to evaluate CAR T cells for solid tumors in the presence of an ECM, which is relevant for modeling solid tumors. In general, impedance was more sensitive than fluorescence measurements and was less prone to large standard deviations.

References

¹ Helsten T. et al. Clin Cancer Res. 2016 Jan 1;22(1):259-67.
² Wu DQ et al. J Biol Chem. 1991 Sep 5;266(25):16778-85.