

Abstract

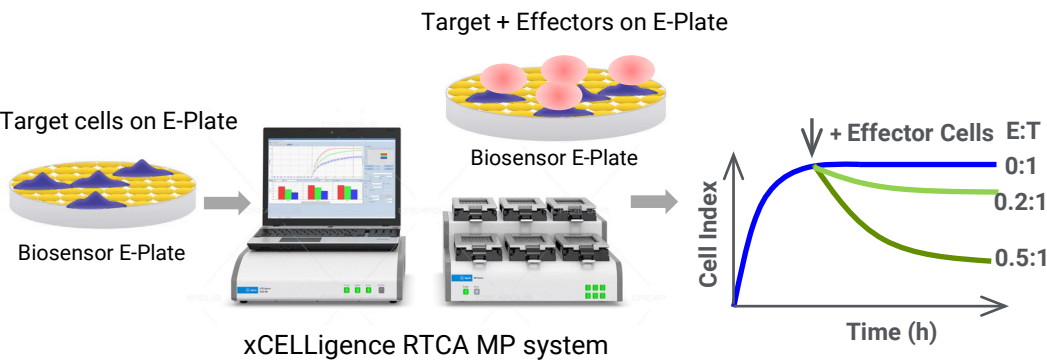
Cancer immunotherapy empowers the immune system to recognize and destroy cancerous cells specifically. Though designed to specifically kill cancer cells, side effects such as cytokine storms and on/off-target toxicity towards normal tissue/organs remain considerable safety concerns that can be difficult to predict using animal models. Bispecific T cell Engagers (BiTEs) are engineered antibodies where two monoclonal antibodies' single chain variable fragments (scFvs) are artificially fused together. One scFv is specific for the target antigen expressed on cancer cells and the opposing scFv targets the CD3 receptor on T cells. These BiTE antibodies facilitate the immune system's interaction with the tumor cells. To explore the potential on/off target immune-mediated toxicity of EpCAM-CD3 BiTE (EpCAM-BiTE) antibodies, a panel of human primary cells representing different tissues was exposed to EpCAM BiTE in the presence of PBMCs and monitored in real time through the impedance measurement of the Agilent xCELLigence RTCA system. Our data shows that EpCAM BiTE did mediate PBMC-induced cytotoxicity of different cell types in a manner that correlated with the expression level of EpCAM. Importantly, an irrelevant BiTE, CD19-BiTE, whose target is not expressed in the primary cells did not induce significant PBMC-mediated cytotoxicity. A dose-dependent decrease in the viability of the primary cells demonstrated the PBMC toxicity mediated through EpCAM BiTE. Overall, our data suggest that using human primary cells combined with the impedance readout can effectively evaluate reagents' on/off-target toxicity, thereby ensuring a safe BiTE product for cancer immunotherapy.

Experimental Design

Target cells: cancer cell lines T-47D and HeLa (both from ATCC); primary normal human keratinocyte (NHEK), normal human astrocyte (NHA), and normal human renal proximal tubule epithelial cells (Lonza).

Effector cells: cryopreserved normal human PBMC (ixCELL), recovered and cultured for 2 days.

Workflow:



Results and Discussion

The level of EpCAM expression in primary cells and cell lines

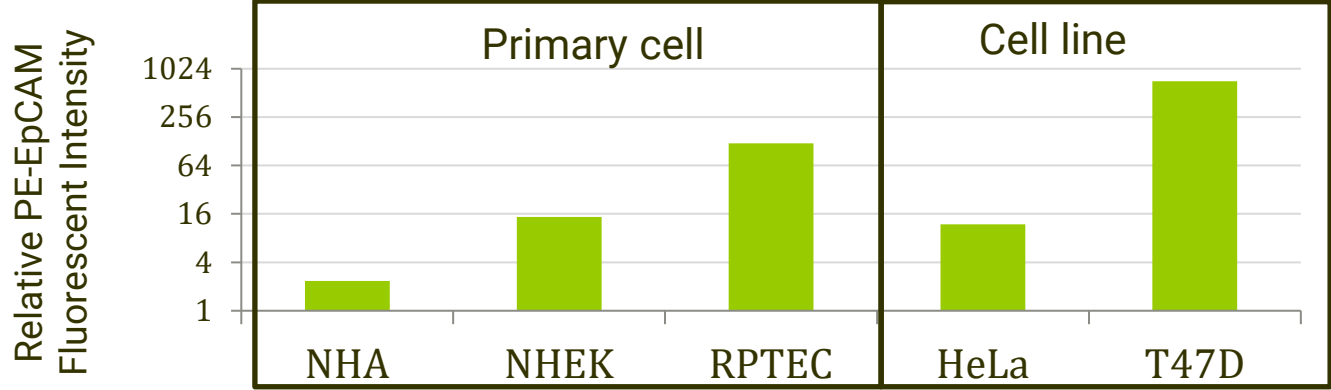


Figure 1. Cell surface EpCAM expression level in primary cells and cell lines. PE-anti-EpCAM was detected on an Agilent NovoCyte flow cytometer.

EpCAM-BiTE facilitates PBMC-mediated-cytotoxicity against cancer cell lines with high EpCAM expression

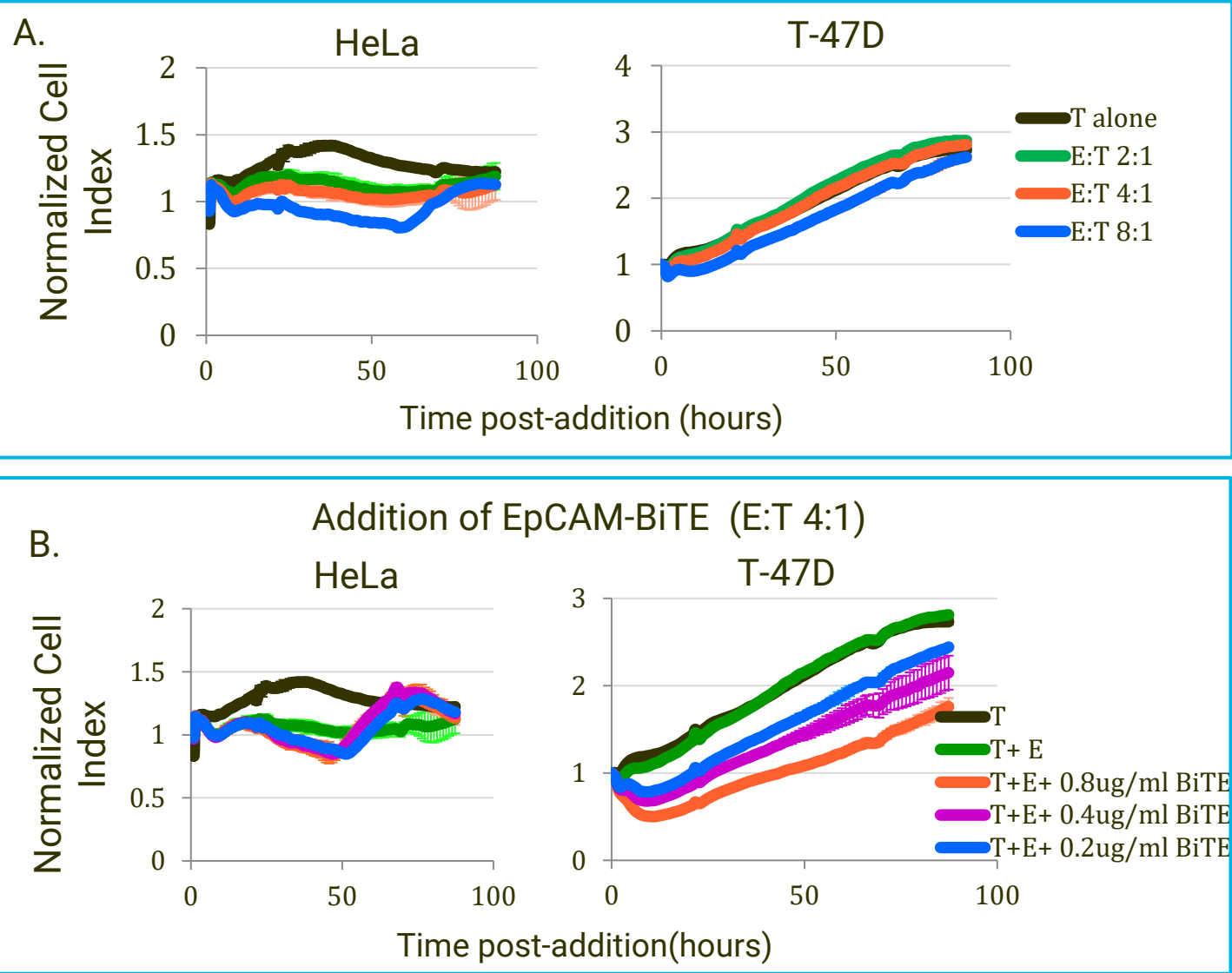


Figure 2. Cytotoxicity activity of PBMCs in the absence and the presence of EpCAM BiTEs. HeLa and T-47D cells grew in E-Plate 96 overnight. They were then treated with activated PBMCs at E:T ratios of 2:1, 4:1, and 8:1 (A); and were treated with activated PBMCs at E:T ratio of 4:1 in the presence of EpCAM BiTEs at the final concentrations, 0.2, 0.4, and 0.8 µg/mL (B).

References

1. Haanen JBag et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up Annals of Oncology, 28 (4), 119–142.

Results and Discussion

Normal human primary cells showed different sensitivities to EpCAM BiTE-mediated cytotoxicity of PBMCs

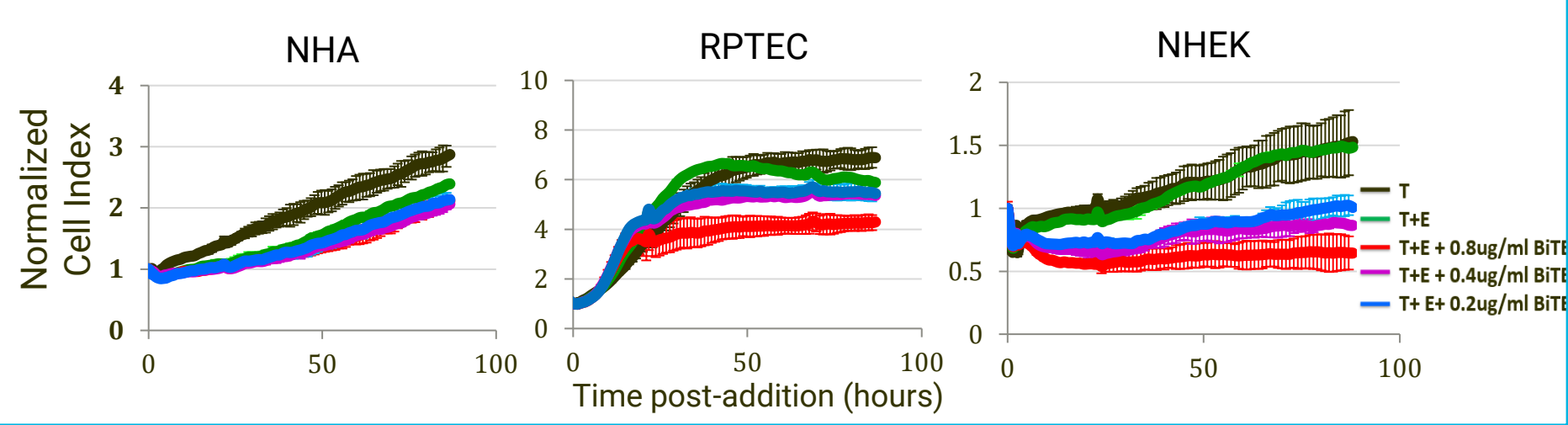


Figure 3. The impacts of EpCAM-BiTE on cytotoxic activity of PBMCs. Primary cells, NHA, RPTEC, and NHEK were treated with activated PBMCs at E:T ratio of 4:1 with different concentrations of EpCAM-BiTE.

CD19-BiTE had no effects on the cytotoxicity activity of PBMCs in the cells expressing EpCAM but lacking CD19

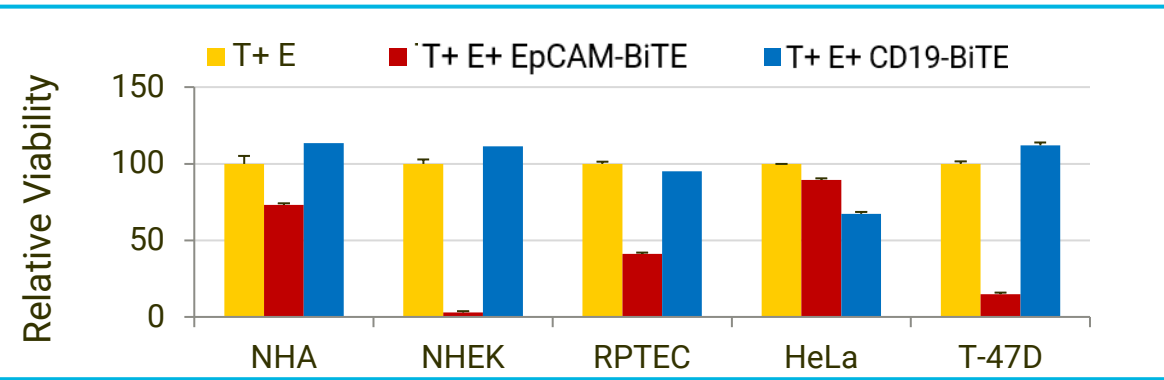


Figure 4. The impacts of BiTEs on cytotoxicity of PBMCs in the tested cells. The relative viability was evaluated at 24 hours post-addition of PBMCs and BiTEs, calculated as NCI of treated wells compared to the target cell and effector cell wells (T+E).

Summary of off-target effect & clinical side effect

Target	Affected patient ³	Onset in patient ¹	Cell model (human primary cells)	EpCAM expression	EpCAM-BiTE cytotoxicity
Dermatological	30-50%	3-4 weeks after treatment	NHEK	Medium	Yes (++)
Kidney	1 to 5%	3 months after treatment	RPTEC	High	Yes (+)
Neurological	1-3%	Rare, but can be fatal.	NHA	low	No (-)

Conclusions

- Impedance readout of Agilent xCELLigence RTCA can capture and quantify BiTE-specific tumor-killing activity in real time.
- The off-target effects of BiTE can be detected and identified using a human primary cell panel.
- The EpCAM-BiTE-mediated immune cell killing activity is related to cell surface EpCAM expression level.
- The on/off-target effects measured using impedance-based technology showed a better correlation to side effects in different tissues/organs than surface marker expression analysis.
- Time dimension correlates and may help to predict the onset and progress of drug effect.