Cell therapy assays

Time-resolved analysis of CAR-T Cell induced apoptosis using an image-enhanced flow cytometer enabled by BrightComp eBeads

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Abstract

In the context of Chimeric Antigen Receptor T-cell (CAR-T) research, understanding the dynamic interactions between CAR-T cells and target cells is essential for optimizing cytotoxic efficacy. This comprehensive methodology, employed in our study aims to provide an end-to-end solution to gain a deeper understanding of CAR-T cell and target cell interactions, particularly in terms of apoptosis induction. The findings from this study can contribute to the optimization and development of CAR-T cell models.

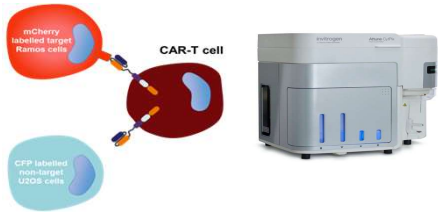
Introduction

The analysis of CAR-T cell interaction with dimly expressed fluorescent protein in target cells presents a major compensation challenge especially amidst high numbers of non-target cells. Further, very short and transient interaction of the CAR-T cell with the target cell, makes it more challenging to observe and characterize various CAR-T cell candidates for cell-cell interactions.

The Invitrogen™ BrightComp eBeads™ fluorescent protein compensation particles can be used when compensating for samples expressing fluorescent Proteins. Using beads rather than cells for compensation is convenient and even necessary in many cases where there is very low percentage of fluorescent protein expression and in order to save precious samples. Invitrogen™ Attune™ CytPix™ Flow Cytometer is an invaluable tool in this workflow which not only offers a quick and robust multiparameter flow cytometry analysis capabilities, but with the combination of a bright field camera and an AI enabled image-analysis platform enables complex interaction studies that were previously impossible to study.

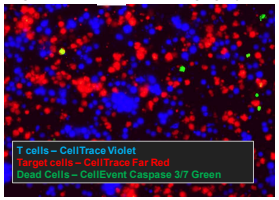
Materials and methods

Figure 1: Analysis of antigen-dependent killing of tumor cells by CAR-T cells using image-guided flow cytometry



1. Label CAR-T cells with Invitrogen™ CellTrace™ Far Red stain
2. mCherry labelled Ramos cells were target
3. CFP labelled U2OS cells as non-target
4. Mix cells 1:1:1
5. Incubate 1 – 4 hours
6. Add Invitrogen™ CellEvent™ Green Caspase-3/7 Reagent
7. Add Invitrogen™ SYTOX™ AADvanced™ dead cell stain
8. Analyze by Invitrogen™ Attune™ CytPix™ Flow Cytometer

Imaged in EVOS™ M7000 Imaging System



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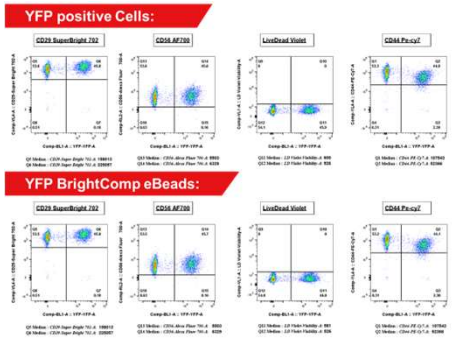
Results

Figure 2: BrightComp eBeads can perform as well as cells in compensating for fluorescent proteins in a multi-parameter flow cytometry experiment



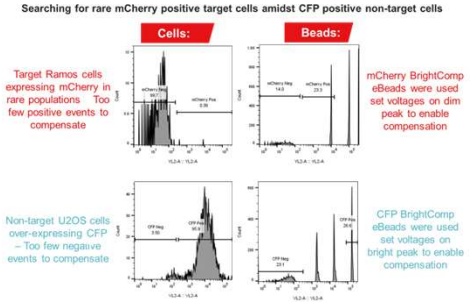
5 Color panel compensated using YFP expressing U2OS cells or YFP BrightComp eBeads

YFP Median Ratio

Costain	Cells	median	Ratio	Beads	median	Ratio
CD56 AF700 (RL2)	Negative	5503	0.87	Negative	5503	0.87
	Positive	6329		Positive	6329	
CD29 SB702 (VL4)	Negative	158013	0.70	Negative	158013	0.70
	Positive	225057		Positive	225057	
Live Dead Violet (V12)	Negative	581	1.10	Negative	600	1.14
	Positive	526		Positive	526	
CD44 PE Cy7 (YL4)	Negative	107543	2.05	Negative	107543	2.05
	Positive	52366		Positive	52495	

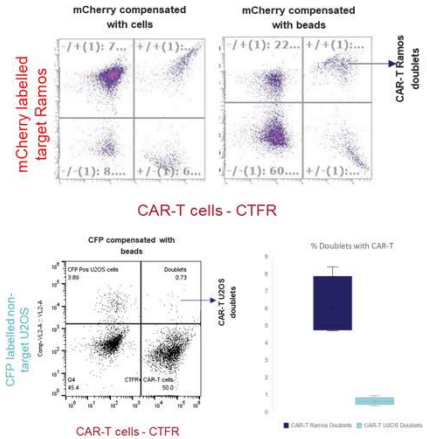
YFP labelled U2OS cells were labelled with 3 different surface co-stains and 1 viability stain and the 5 color panel was compensated using either YFP labelled cells or YFP BrightComp eBeads showing similar separation and populations

Figure 3: BrightComp eBeads enabled compensation of fluorescent proteins in cell systems with low positive or low negative target populations



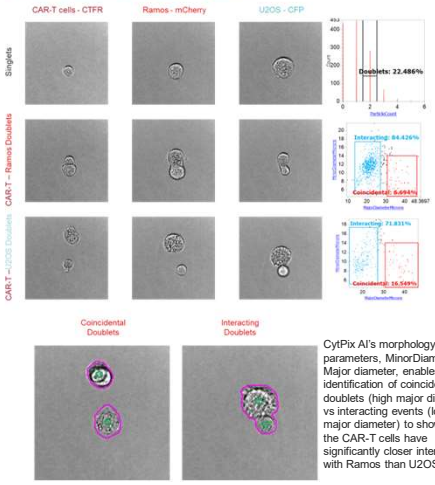
- Target Ramos cells had too few mCherry positive rare cell events that were of interest– 0.3% positive. It was not possible to use it for compensation. Hence mCherry BrightComp eBeads offer a suitable alternative to set voltage and compensation. The dimmest of the 3 peaks to the mCherry brightness of my positive populations
- Similarly, in the non-target U2OS cells, we observed >96% CFP positive cells. It was not rationally possible to gate the low CFP expressing cells as true negatives. So it BrightComp eBeads can be used for compensation again.

Figure 5: Distinguishing CAR-T cell interaction with mCherry positive target cells vs CFP positive non-target cells



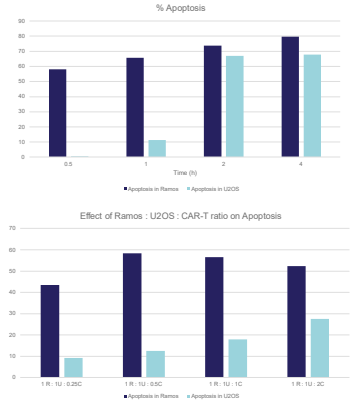
Co-culture of CFP labelled U2OS; mCherry labelled Ramos and Cell trace Far Red labelled CAR-T cells were analyzed using an Attune CytPix flow cytometer and the formation of immune synapse between the target cancer cells and the CAR-T cells was analysed.

Figure 5: Attune CytPix's advanced image analysis AI is capable of distinguishing coincidental doublets from interacting doublets



CytPix AI's morphology parameters, MinorDiameter vs Major diameter, enables identification of coincidental doublets (high major diameter) vs interacting events (low major diameter) to show that the CAR-T cells have significantly closer interaction with Ramos than U2OS

Figure 5: CellEvent Green Caspase-3/7 Reagent enables study of CAR-T induced tumor cell apoptosis without disrupting cell-cell interactions



Our analysis showed that the CAR-T cell induced apoptosis increased over-time on target Ramos cells but beyond 2 h there was a lot of death observed in non-target U2OS cells. Further we observed a lower Ramos:CAR-T cell ratio of 1:0.5 resulted in better tumor cell specific cell kill as against higher ratios of CAR-T cells.

Conclusions

This comprehensive methodology, employed in our study aims to facilitate a deeper understanding of CAR-T cell and target cell interactions, The ability to visualize cell-cell interactions between CAR-T cells and their target cells on the CytPix and quick low intervention apoptosis assay opens new ways to measure and confirm the efficacy of CAR-T induced cell death for an optimized CAR-T development and testing platform.

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