Developing image-based T-cell assays to effectively monitor apoptosis and CAR expression

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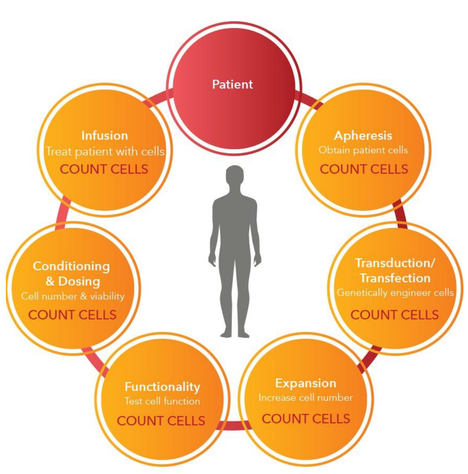
1. Abstract

Chimeric antigen receptor (CAR)-T cell therapy is a novel cellular therapeutic approach for cancer patients, including B-cell malignancies. A critical step in the CAR-T cell production process is to effectively deliver CAR genes into primary T- cells, which can be achieved through the use of viral vectors or non-viral methods. To evaluate and select effective CAR gene delivery methods and processes, it is imperative to perform analytical tests to detect and monitor cell proliferation, cell health status, and CAR gene expression. In this work, we developed an image-based method using the Cellaca® PLX Image Cytometer to quickly count T-cells, measure viability, assess apoptotic cell health, and identify CAR expression. Using this new methodology, we compared different CAR gene delivery methods, primarily focusing on non-viral methods involving electroporation. Cell viabilities were monitored daily using acridine orange / propidium iodide (AO/PI) stain and its respective dual fluorescent assay. Preliminary results showed that viabilities for all SupT1 samples decreased significantly to ~50% by day 1 following electroporation, in comparison to un-transduced SupT1 control samples, which maintained ~90%+ viabilities. These results confirmed that the introduction of plasmids, rather than the electroporation process itself, induced apoptosis and eventually cell death. Additionally, Annexin V / PI and Caspase-3 / RubyDead cell health assays were tested, and results indicated that a majority of cell death following electroporation was likely the results of apoptotic cells transitioning to the point of no return – cell death. Transduced SupT1 samples were able to fully recover, as their viabilities increased to ~90%+ by day 5 of the study. Lastly, samples were stained with APC-conjugated specific anti-CAR antibody, and SupT1 CAR expression levels were measured using the Cellaca® PLX, and results were confirmed using a flow cytometer. Utilizing this image-based method, we were able to monitor CAR expression in SupT1 cell samples on day 2, 5, 7 and compare CAR expression levels among different gene delivery methods (viral vectors or non-viral methods). With the advantages of ease of use, visual verification with captured cell images, and higher-throughput capability, the Cellaca® PLX Image Cytometer may be potentially used as a convenient benchtop system for rapid assessment of the quantity and quality of CAR-T cells, which may ultimately improve the productivity of development and manufacturability of CAR-T products.

2. General process of CAR-T cell production

Chimeric antigen receptor (CAR) **T** cell therapy involves the engineering of T-cells to attack tumor cells by specifically binding to tumor antigens.

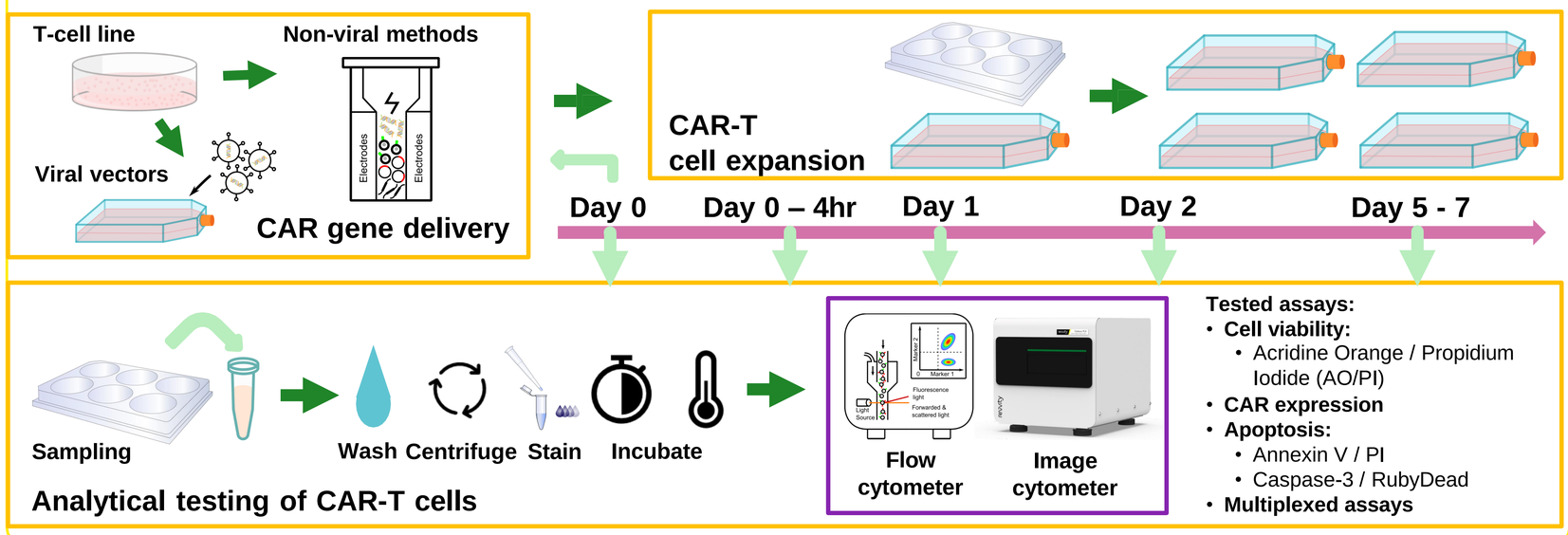


CAR gene delivery methods

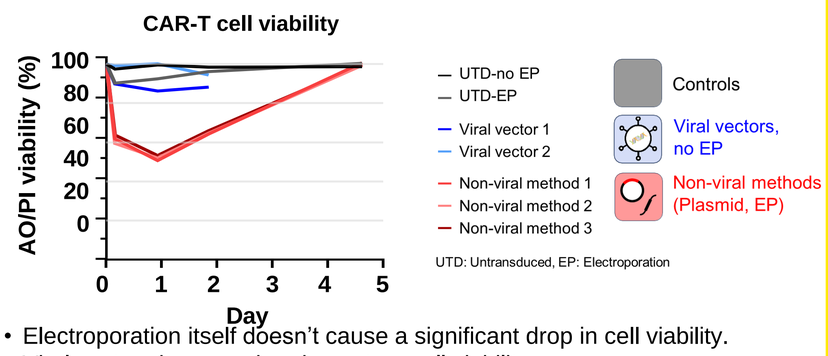
Viral vectors
- Retroviral vectors (e.g., AAV)
- Lentiviral vectors

Non-viral methods
- Plasmid
- mRNA

3. Workflow for CAR gene delivery, cell expansion and characterization



4. CAR-T cell viability results with premixed duo fluorescent AO/PI dyes



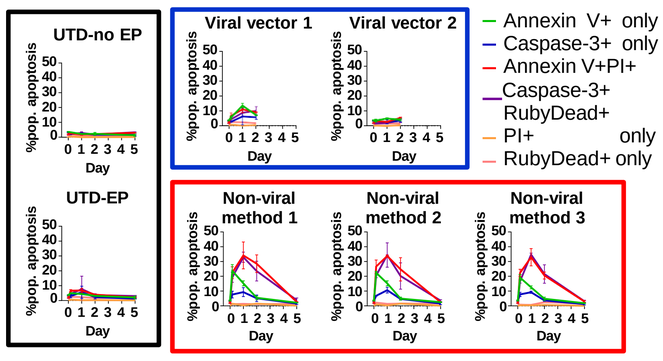
- Electroporation itself doesn't cause a significant drop in cell viability.
- Viral vectors have a minor impact on cell viability.
- Cell viability using non-viral methods drops to ~60% at ~4 hours and ~50% by day 1.

5. Monitoring apoptosis using Annexin V / PI and Caspase-3 / RubyDead assays

Annexin V: Detects early-stage apoptosis

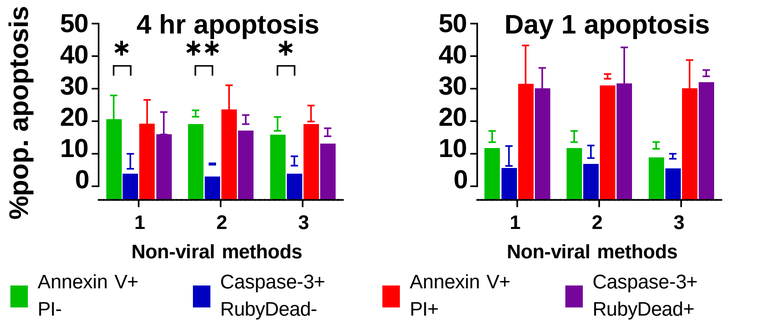
Caspase-3: Detects late-stage apoptosis

PI or RubyDead: Detects dead cells



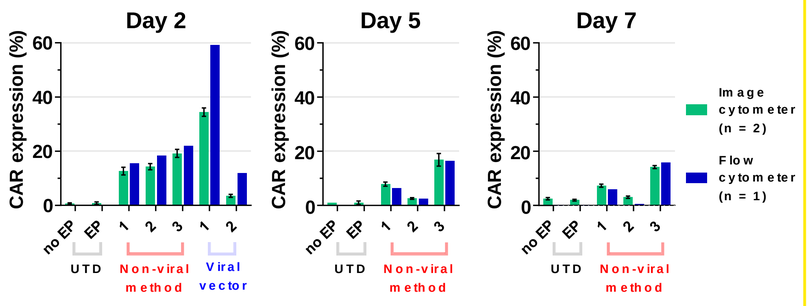
- Electroporation (EP) causes a small increase in apoptosis (~10%).
- Introducing a plasmid or a viral vector may induce significant apoptosis.

6. Transition from early- to late-stage apoptosis, and eventually cell death



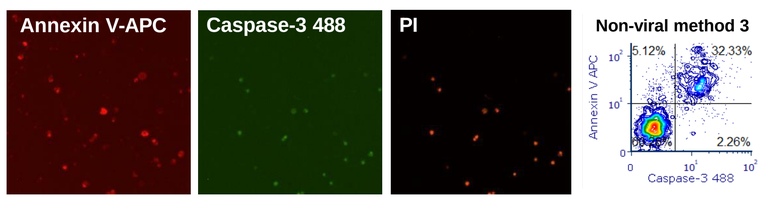
- At ~4 hours, the difference in the % population between early- and late- stage apoptotic cells is ~15%.
- On day 1, the early-stage apoptotic cell population drops, while the late- stage apoptotic and dead cell populations increase.

7. Identification of CAR expression



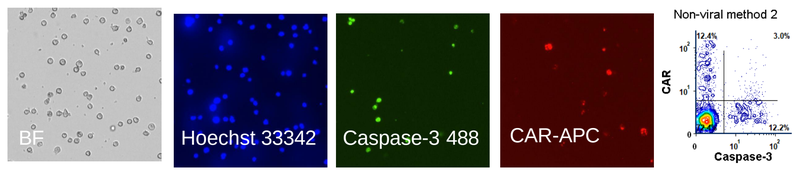
- CAR expression results between image and flow cytometers are comparable.
  - Except for viral vector 1 and 2 on day 2.

8. Multiplexed apoptosis assay



- Multiplexed apoptosis assay can provide an additional examination on whether the Annexin V+ cells are also Caspase-3+.

9. Multiplexed CAR expression and apoptosis



- Multiplexed CAR expression and apoptosis assay can detect the percentage of CAR+ cells that are or are not late-stage apoptotic.

10. Summary

- Non-viral methods for CAR gene delivery resulted in a significant decrease in cell viability following electroporation.
- Most cells entered the apoptotic pathway following electroporation, eventually entering the point of no return – cell death.
- CAR expression can be characterized using image cytometry, with results confirmed through flow cytometry.
- Image cytometry based multiplex assays can provide rapid measurements for the following: direct cell counting, viability, apoptosis, and CAR expression

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