Protocol 1012

AGILEPULSE™ MAX ELECTROPORATION PROTOCOL

Cell Line: T lymphocytes

Application or Transfectant: mRNA

Electrode: 4 mm gap Cuvette Item# 45-0126 or 4 mm gap 10 ml Flatpack Item# 47-0206

Cell Preparation:

Primary T lymphocytes isolated from PBMC were activated for 3 to 6 days prior to electroporation. Activation beads were removed, then cells were pelleted and resuspended in electroporation buffer.

Group 1 Electroporation Settings:

Set Voltage:	1200 V
Set Pulse Length:	0.1 ms
Set Number of Pulses:	1
Set Interval Time:	0.2 ms
Desired Field Strength:	3000 V/cm

Group 2 Electroporation Settings:

Set Voltage:	1200 V
Set Pulse Length:	0.1 ms
Set Number of Pulses:	1
Set Interval Time:	300 ms
Desired Field Strength:	3000 V/cm

Group 3 Electroporation Settings:

Set Voltage:	130 V
Set Pulse Length:	0.2 ms
Set Number of Pulses:	4
Set Interval Time:	2 ms
Desired Field Strength:	325 V/cm

Electroporation Procedure:

Electrode Gap:	4 mm gap cuvette, Item # 45-0126
Total Sample Volume:	200 µl
Number of Cells:	5 x 10^6 cells/ml
Electroporation Buffer:	Cytoporation Medium T (Item # 47-0002)
Amount of Transfectant:	30 µg mRNA

Pulse:	Press Load for system to automatically estimate load resistance. Tap Ready button when it turns yellow to allow system to charge. Tap Start button when it turns yellow to deliver pulse.
Post Treatment:	Cells were diluted into 2 mL culture medium and incubated at 37°C/5% CO2.

Note: Please use ONLY Cytoporation T buffer for this application

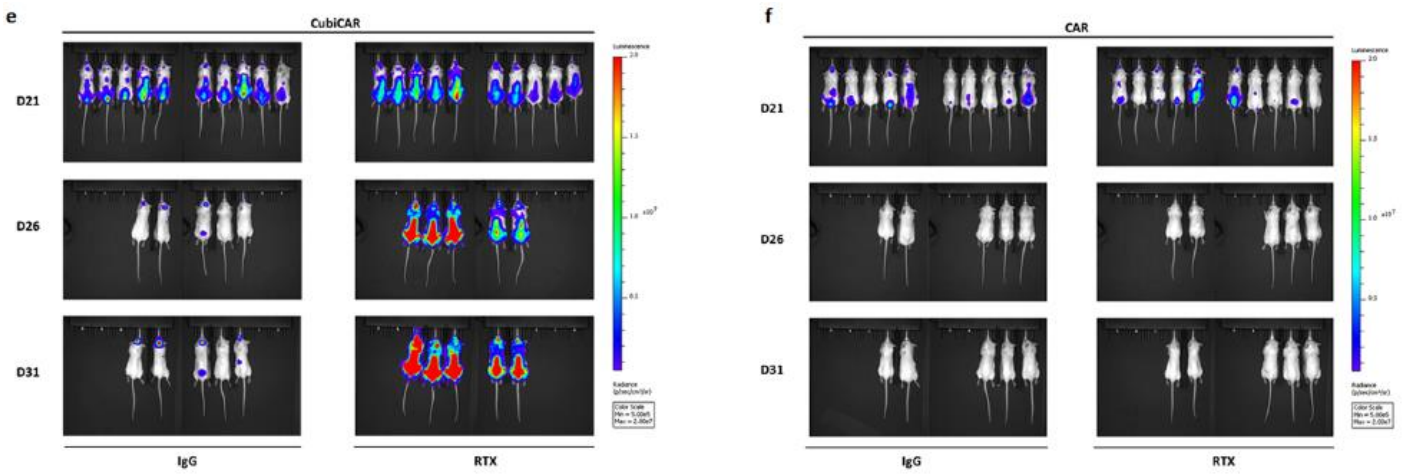
Results:

Valton et al., (2018) describe a trifunctional Chimeric Antigen Receptor architecture which includes a therapeutic molecule that makes it easier for CAR T-cells to be detected, purified, and silenced by an on-demand suicide switch. The transfection efficiency was measured by flow cytometric detection of transient expression of CAR constructs on the surface of primary T-cells and the viability was determined by the ability of CAR constructs to promote T-cell depletion by cytotoxic assay.

Efficiency: 70 to 90%

Viability: 30 to 100%

Immunodeficient BRGS mice, deficient in T, B, and NK cells were used as models to test the in vivo anti-tumor activity and depletability of the CubiCAR T-cells. Bioluminescence live imaging of mice and flow cytometry analysis of the mice blood, bone marrow and spleen were used to monitor tumor expansion as well as proliferation and Rituximab-mediated depletion of mock transduced, CAR, and CubiCAR T-cells. Efficient anti-tumor activity of CAR and CubiCAR T-cells was confirmed by the reduction of the bioluminescence signal over time. In contrast, injection of RTX in mice treated with CubiCAR T-cells resulted in an unchecked proliferation of tumor cells as demonstrated by a high bioluminescence signal.



References:

Valton J *et al*., A versatile safeguard for chimeric antigen receptor T-cell immunotherapies. Nature 2018; 8: 8972.

Juillerat, A. et al., Design of chimeric antigen receptors with integrated controllable transient functions. Nature 2016; 6: 18950.

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