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Haute Ecole Spécialisée de Suisse occidentale
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Master of Science HES-SO in Life Sciences

Enhancement of Chimeric Antigen Receptor (CAR) Cell Therapy by Modification on Tumor Microenvironment

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DESCRIPTION

Cancer is on of the main causes of death worldwide. In Switzerland, it is the second disease causing death after cardiovascular diseases with 16'930 people dead.[1] Most common types of cancers are breast (12.2%), prostate (11.1%), colorectum (7.6%), lung (7.5%), melanoma (5.6%) and others (56.2%).[2] Cancer is subject of intensive research to develop new curative approaches. Treatment against blood malignancies, has shown encouraging results using cell-based therapy. Investigation to transpose this approach for use in solid cancer is in continuous progress.

Tumor microenvironment (TME) in solid tissues are associated with hostile conditions to immune cells function. This project aims to combine the use of cell therapy, namely T lymphocytes expressing chimeric antigen receptor (CAR T-cell), with local release of a specific enzyme intended to mitigate TME harsh conditions. CAR molecule is composed of: an antibody derived recognition domain, transmembrane domain, co-stimulatory and activation domains. Expression of the enzyme in our setup is linked to the cell activation via the CAR signalling pathway. Cell activation induce the recombinant enzyme expression which perform dismutation on reactive oxygen species (ROS) and release oxygen as product. Increasing O_2 concentration, counteracts hypoxia environment and reduce acidic niches. As consequence, the ability of cancer cells to grow, persist and survive are reduced. In addition, it provides to the immune system better condition to improve the effectiveness of anti-tumor activity.

In this study, Jurkat cells and primary $CD4^+$ lymphocytes were transfected with different DNA plasmids to induce extracellular recombinant enzyme production. Expression of the protein gene in these constructions was linked to the minimal or natural IL-2 promotor, and three or six nuclear factor of activated T-cells (NFAT) response elements. Secretion of the protein was driven by CD8 or IL-2 signal peptide. The expression positive control was tested using the plasmid with EF-1α promoter.

1. https://www.bfs.admin.ch/bfs/en/home/statistics/health.assetdetail.24131868.html
2. https://gco.iarc.fr/today/data/factsheets/populations/756-switzerland-fact-sheets.pdf

OBJECTIFS

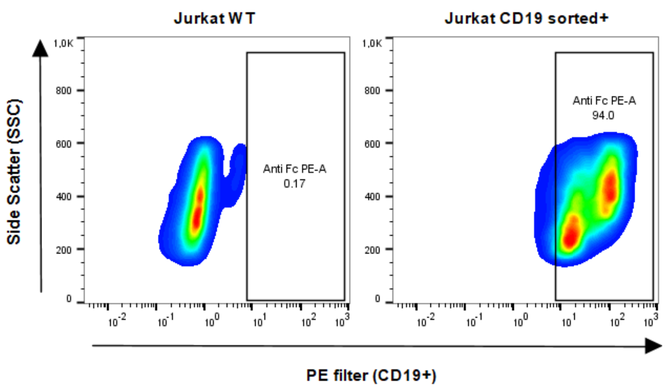
- Design planned DNA sequences of our interest into pUC18 plasmids.
- Clone the DNA sequences into pSBbi plasmids with restriction enzyme.
- CAR-CD19 detection on genetycally modified Jurkat by flow cytometer.
- Measure IL-2 cytokine release on Jurkat cells and T lymphocytes using several stimulation methods: PMA-Ionomycin, beads conjugated with anti-CD3/CD28 antibodies, cocktail made of anti-CD28 and anti-CD3, or rhCD19 protein.
- Detect enzyme activity by colorimetric assay on plasmid-transfected Jurkat and T cells previously stimulated.

RESULTS

CAR-CD19 detection on Jurkat membrane

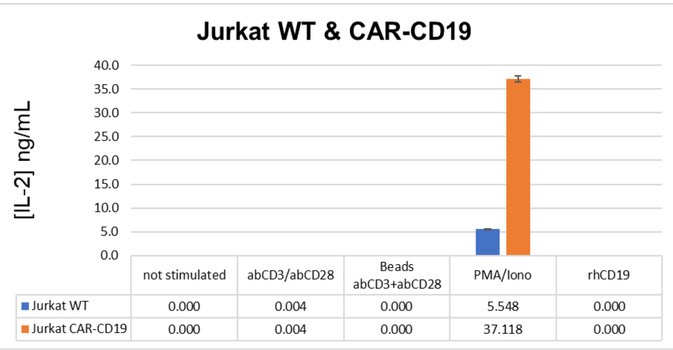
Jurkat cells were incubated with recombinant human CD19 protein-Fc followed by anti-human IgG Fcy fragment specific Phycoerythrin (PE) conjugated and analysed to flow cytometer (graph beside).

The result has shown that 94% of the population have expressed the CAR against less than 1% for the negative control Jurkat WT. In the right graphic, two positive populations are clearly visible. This is due to the entire population do not coming from a clone but from two sorting procedures.

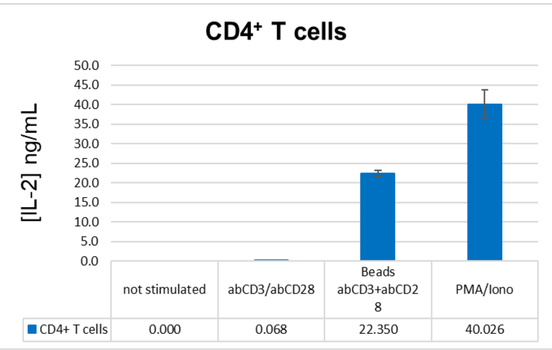


IL-2 cytokine detection by ELISA

Jurkat WT and CAR-CD19 were cultured unstimulated or stimulated with rhCD19 protein or with a cocktail of anti-human CD28 and anti-human CD3 antibodies, or with beads conjugated with in anti-human CD28 and anti-human CD3 antibodies, or with PMA/Ionomycin. $CD4^+$ T cells were tested as the Jurkat but without rhCD19 stimulation.



	not stimulated	abCD3/abCD28	Beads abCD3+abCD28	PMA/Iono	rhCD19
Jurkat WT	0.000	0.004	0.000	5.548	0.000
Jurkat CAR-CD19	0.000	0.004	0.000	37.118	0.000

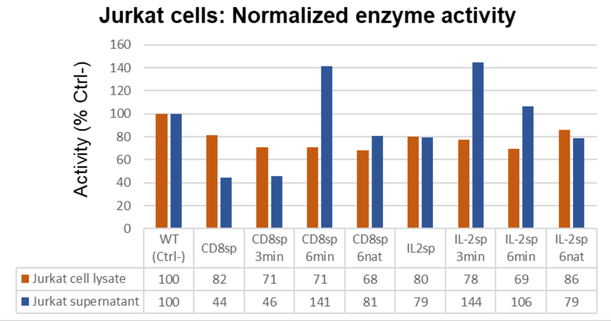


	not stimulated	abCD3/abCD28	Beads abCD3+abCD28	PMA/Iono
CD4+ T cells	0.000	0.068	22.350	40.026

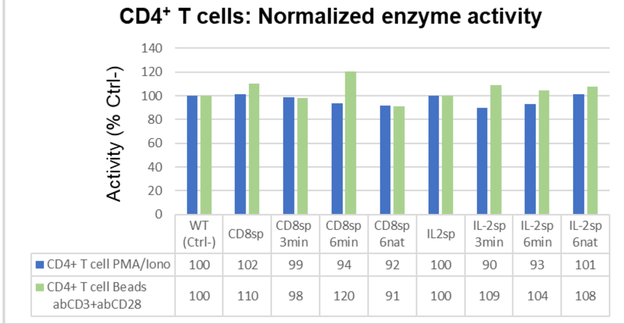
Jurkat showed no secretion of IL-2 cytokine once stimulated with the rhCD19 protein, indicating the intracellular signaling was not efficient, or not in high intensity enough, to activate Jurkat cells. Jurkat CAR-CD19 were not more tested for the folllowing experiments. Stimulation with PMA and Ionomycin for both cell populations showed high amounts of IL-2 in every test. Beads conjugated showed also superior performance in stimulating primary $CD4^+$ T cells.

Enzyme colorimetric activity determination

Once the tested plasmids were normalized on negative control, none of them goes higher than negative control (100%) for the lysate conditions but only three have exceeded for the cells supernatant conditions with, +6%, +41%, +44% of activity. For T cells, beads stimulation has led to low recombinant enzyme production for five plasmids.



	WT (Ctrl-)	CD8sp	CD8sp 3min	CD8sp 6min	CD8sp 6nat	IL2sp	IL-2sp 3min	IL-2sp 6min	IL-2sp 6nat
Jurkat cell lysate	100	82	71	71	68	80	78	69	86
Jurkat supernatant	100	44	46	141	81	79	144	106	79



	WT (Ctrl-)	CD8sp	CD8sp 3min	CD8sp 6min	CD8sp 6nat	IL2sp	IL-2sp 3min	IL-2sp 6min	IL-2sp 6nat
CD4+ T cell PMA/Iono	100	102	99	94	92	100	90	93	101
CD4+ T cell Beads abCD3+abCD28	100	110	98	120	91	100	109	104	108

CONCLUSION

- CAR-CD19 on genetically modified Jurkat cells is expressed but did not activate them to release IL-2 once stimulated with rhCD19.
- PMA/Ionomycin and beads anti-CD3 and anti-CD28 conjugated are efficients stmulation molecules for T cells. Only PMA/Ionomycin provide efficient activation for Jurkat cells population.
- Detection of active recombinant enzyme was lower than expected. Colorimetric measurments for the enzyme showed marginal activity for pSBbi-CD8sp-6min and pSBbi-IL-2sp-3min plasmids.
- There was no evidence that secreted enzyme activity was superior with the signal IL-2 peptide than with the CD8 signal peptide.
- No evidence of recombinant enzyme expression was detected. Possible causes: low transfection efficacity for T-cells and several mutations located in promoters (data not shown), further investigations are expected to be done.
- Additional detection methods should be tested such as: at translation level, protein immunodetection tests like Western Blot or ELISA, and at the transcription level with mRNA detection using real-time PCR test. Positive control cell population should be created with the positive control plasmid coding for the enzyme in order to verify if the coding sequence is functional. Another factor is the cell line, Human embryonic kidney (HEK-293) cells are a well established line for protein expression and is currently being tested.

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