MARTA WĘGIERSKA, MSC
DEPARTMENT OF TUMOR BIOLOGY
Marta.wegierska@stud.umed.lodz.pl

International Doctoral School
Medical University of Lodz

ASSESSMENT OF ANTI-CD19 AND ANTI-CD22 CHIMERIC ANTIGEN RECEPTORS EXPRESSION REGULATION SYSTEM IN T CELLS

• INTRODUCTION

CAR-T cell therapy is more often used to treat patients with leukemia and lymphoma. It results in prolonged remission in some cases but can also induce cytotoxicity and lead to loss of the antigen that is targeted [1]. New approaches are needed in CAR-T cell therpies to **reduce cytotoxic effect** in patients and to **avoid molecular changes** in cancer cells hampering further treatment [2].

Two different approaches were developed so far: using **bi-specific CAR-t cells** and **regulating the expression** of chimeric antigen receptor (CAR) in mono-specific CAR-T cells [2]. Several clinical trials with bi-specific CAR-T cells are now conducted (i.e. NCT03706547, NCT04303520, NCT04499573) while higher effectivity CAR-T cells that can be regulared was proven by for example Kotter at al. [3].

In this study, the ultimate goal is to **combine both methods** metioned above. Therefore a new chimeric antigen receptor expression regulation system is being introduced. It is expected that the designed system will let **control CARs expression independently** by means of chemical compounds which are unique for each CAR. For this purpose a mechanism partially based on TET-on/off was designed by means of genetic engineering and tested if it is possible to create such system.

• MATERIALS AND METHODS

The first step was to use two of previousely obtained plasmids containing two separate **transcription factor (TF) binding region and two different reporter gene sequences** (green and red fluorescent protein) to perform viral transduction of HEK293T cells.

Figure 1. Simplified illustration of transcription factor binding sites and transgene located in designed and used plasmids.

Transduced cells were cultured with an addition of antibiotics after each viral transduction to which cells obtained resistance if they adopted the viral vector. After the selection cells were multiplied, sorted based on double-positive fluorescent signal and underwent analysis in presence of chemical compounds that impact the transcription factor binding to the promoter of the fluorescent protein genes.

The activity of tested compounds was analyzed using **fluorescent microscopy**. Green and red fluoresncent protein genes were dependent on the transcription factor binding to its binding site and promoting transgene expression. Chemical compounds being agonists and antagonists of the analyzed transcription factors were used to modulate particular transgene expression. Chemical compounds being agonists and antagonists of the analyzed transcription factors were used to modulate particular transgene expression.

Additional compounds were added to the analysis as potential agonists of one of tested transfription factor. Cells with viral vector used during the analyses were incubated with new chemical compounds and luciferase activity was tested.

• RESULTS

Cytometric and microscopic analyses showed that it was **possible to obtain cellular model with two viral vectors encoding two different transcription factor binding sites with two different transgenes** such as green fluorescent protein (GFP) and red fluorescent protein (dTOMATO). It has to indicated that not all cells have red and green fluorescent signal even after sorting out cells with the highest level of both signals at the same time.

Implementing new chemical compounds which are potent agonists of ABM1 and ABM1/RV complex (RET and PEM) shed a light on **dependencies between compound concentration and luciferase expression regulation effect (Fig 2.)**. RET and RTA increased luciferase activity at both analyzed concentrations while PLZ and PEM which are considered as specific ABM1 agonists elevated luminescence level at concentration of 2.5uM **(Fig. 2A)** but decreased luciferase activity at 10uM **(Fig. 2B)**.

Obtained results show that chemical compounds at the highest tested concentrations that were added during this analysis could have potential toxic effect on tested cells leading to decreased both red and green fluorescent signals and also changed morphology of model cells compared to sample without addition of tested compounds **(Fig. 3)**. The toxic effect can be assosiated with decreased luciferase activity as GFP transgene is located under the same TF bindinf site as luciferase.

Figure 4 presents results of fluorescent microscopic analysis of obtained cell line incubated with addition of one agonist of both ABM1/RV and LRR/RV complexes – RTA, and two antagonists of ABM1 and RV – BXC and H5 respectively. BXC and H5 led to decreased green and red fluorescent signal when compared to the non-treated sample. Morphology and number of these cells also differ from non-treated sample which suggests toxic effect of tested antagonists.

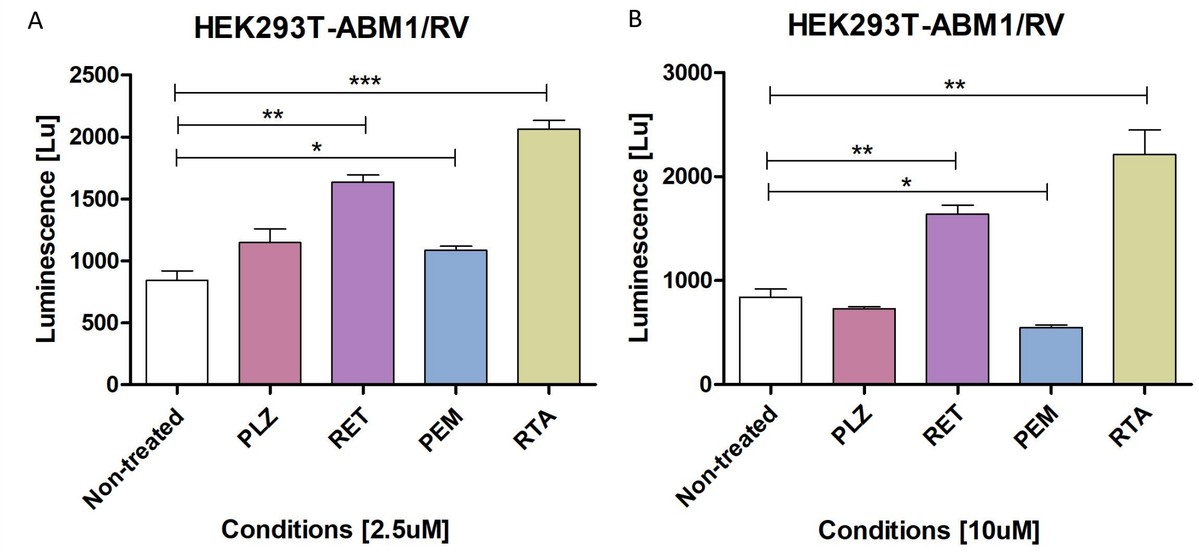

Figure 2. Luminescence levels comparison of cells transduced with viral vectors encoding ABM1 binding site and luciferase gene. Plots present luminescence level corresponding compounds tested at concentration of 2,5uM (A) and 10uM (B).

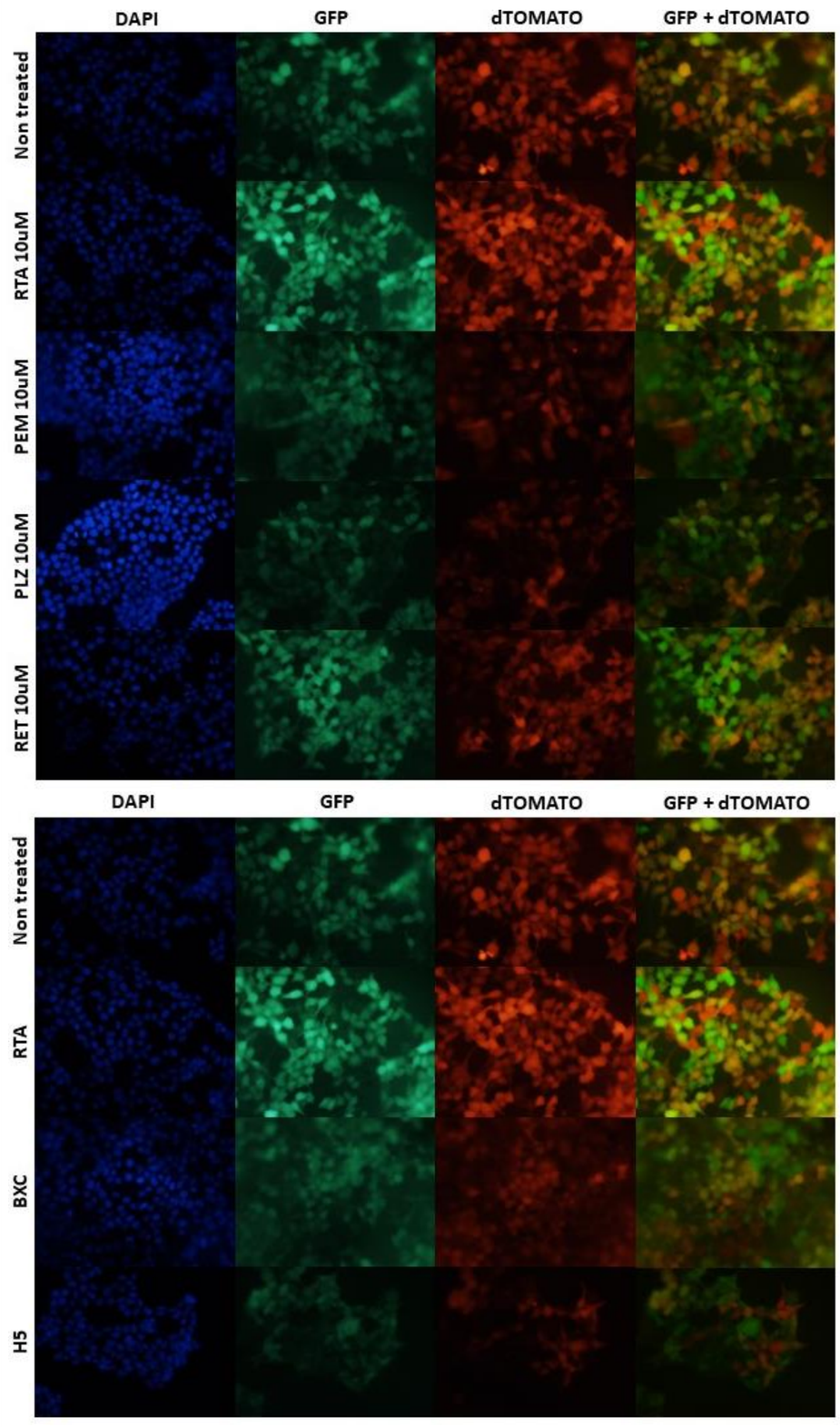

Figure 3. Results of fluorescent analysis of cells transduced with two designed viral vectors encoding ABM1/RV binding site (green) and LRR/RV binding site (red) incubated with new potential agonists of ABM1 and ABM1/RV complex (RET, PEM) compared to already tested RTA and PLZ.

Figure 4. Results of fluorescent analysis of cells transduced with two designed viral vectors encoding ABM1/RV binding site (green) and LRR/RV binding site (red) incubated with RV agonist (RTA) and antagonists of ABM1 and RV (BXC and H5).

• CONCLUSIONS

Obtained results suggest that designed system seems to be **a promissing method** to control an expression of **two different transgenes** using different chemical compounds.
It also has to be pointed out that analysis based on fluorescent signal changes is voulnerable to false negative results while transcription factor expression profile can be different in each cel at one time.
According to the fluorescent signal changes it is **possible to increase expression of each transgene**. Unfortunately chemical compounds expected to decrease transgenes expression levels turned out to have toxic effect on tested cells.
Concentrations at which tested chemical compounds will properly modulate transgene expression while remain non-toxic have to be determined.

• REFERENCES

1. Cappell, K.M., Kochenderfer, J.N. Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol (2023).
2. Gumber D, Wang LD. Improving CAR-T immunotherapy: Overcoming the challenges of T cell exhaustion. EBioMedicine. 2022 Mar;77:103941.
3. Kotter B, Engert F, Krueger W et al. Titratable Pharmacological Regulation of CAR T Cells Using Zinc Finger-Based Transcription Factors. Cancers (Basel). 2021 Sep 22;13(19):4741

MEDICAL UNIVERSITY OF LODZ