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

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• 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 non-xenobiotic drugs 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 design and prepare a second plasmid containing i.e. **transcription factor (TF) binding region and reporter gene sequences**. Obtained plasmid was introduced into bacteria cells through transfection process and then multiplied in bacteria cultured in appropriate conditions as it was performer in case of the first plasmid. Then plasmids were isolated and purified and used to create a lentiviral vector. A properly prepared lentiviral vector was used to conduct eucaryotic cells transduction.

Transduced cells were cultured with an addition of antibiotic to which cells obtained resistance if they adopted the viral vector. After the selection cells were multiplied and underwent analysis in presence of chemical compounds that impact the transcription factor binding to the promoter of the luciferase gene.

The same procedure was performed in case of the third plasmid encoding the **shared part** for both main transgenes being a **necessary part for the system** to work properly.

The activity of tested compounds was analyzed through **luminescence measurements**. Luciferase gene expression was 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.

• RESULTS

Obtained results show the **agonistic and antagonistic effect** of chemical compounds that were chosen for this analysis **(Fig. 1)**. PLZ chosen as ABM1 agonist and 20HC – LRR agonist they both resulted in increased luciferase levels. ABM1 antagonist named BXC caused an icrease of luminescence level **(Fig. 1A)**, while LRR antagonist – GK was able to slightly reduce this signal when compared to the control **(Fig. 1B)**. Combination of agonist and antagonist in case of both analyzed transcription factors led to increased luminescent signal in case of ABM1 (PLZ+BXC) and to decreased signal in case of LRR (20HC+GK).

Figure 2 presents results for both analyzed transcription factor binding sites but each is in combination with the third TF binding site that is a shared part in the mechanism - RV. Additional chemical compounds were applied which are RV agonist and antagonist. Whey were tested in the same manner as previously. RTA - RV agonist was able to increase luminescence levels in cells with ABM1/RV **(Fig. 2A)** or LRR/RV **(Fig. 2B)** vectors, while H5 – RV antagonist gave opposite results. The **most significant changes** in luminescence levels were observed when **both antagonists or both agonists** were used at the same time in case of both combinations.

To enable assessment of the effectiveness of the system in cells using both vectors at the same time it was necessary to introduce two different fluorescent proteins to the cells and the results for green fluorescent protein are shown on the **Figure 3.** The **green signal** was possible to be observed only when cells were **successfully selected** after transfection with viral vector. Transfected cells are compared to cells that were not modified.

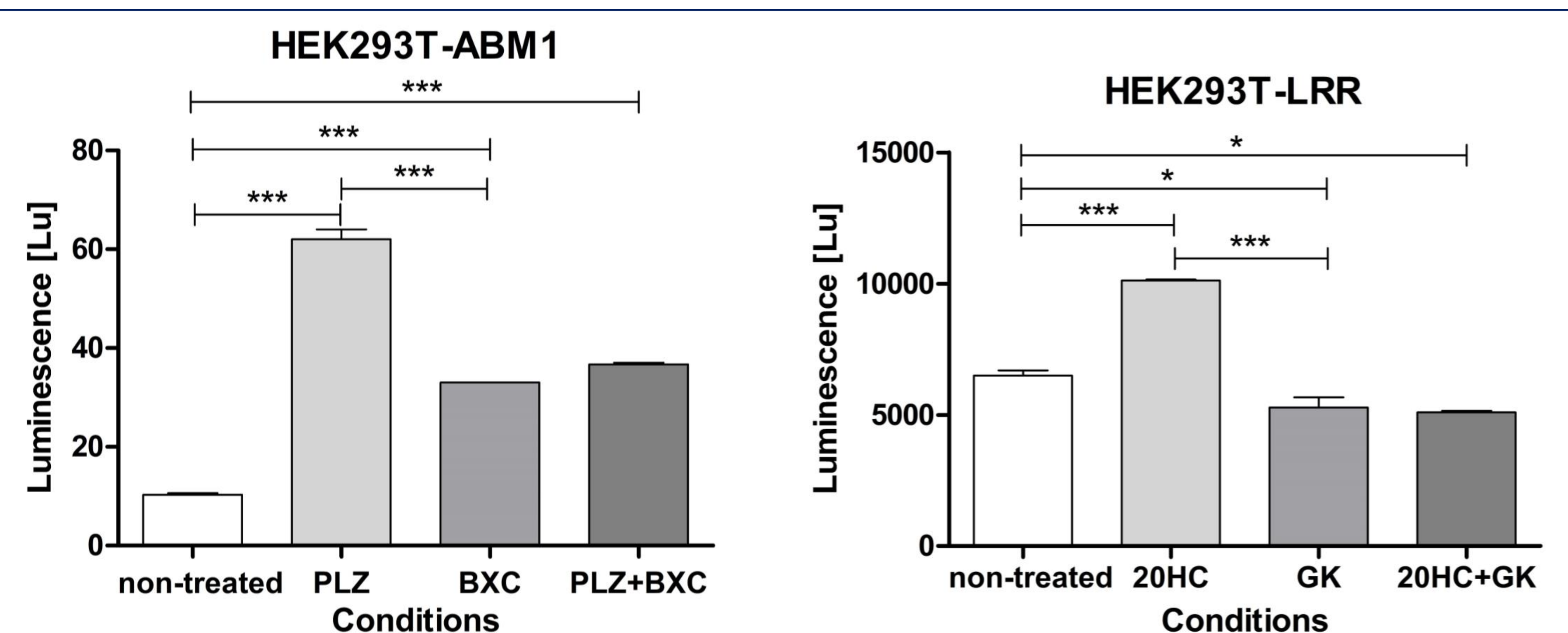

Figure 1. Luminescence levels comparison of cells transduced with viral vectors encoding ABM1 binding site and luciferase gene (A) and LRR binding site and luciferase gene (B).

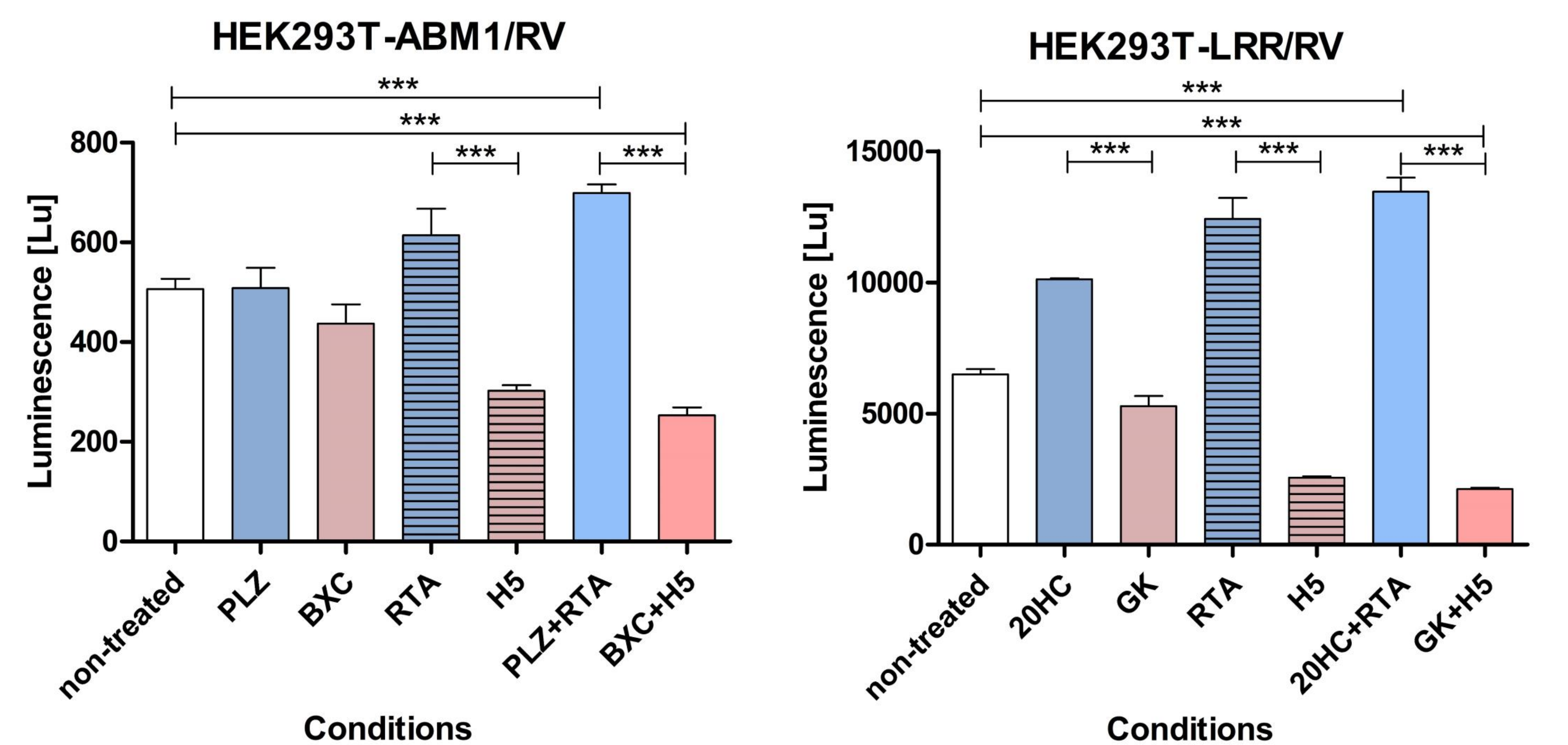

Figure 2. Luminescence levels comparison of cells transduced with viral vectors encoding ABM1/RV binding site and luciferase gene (A) and LRR/RV binding site and luciferase gene (B).

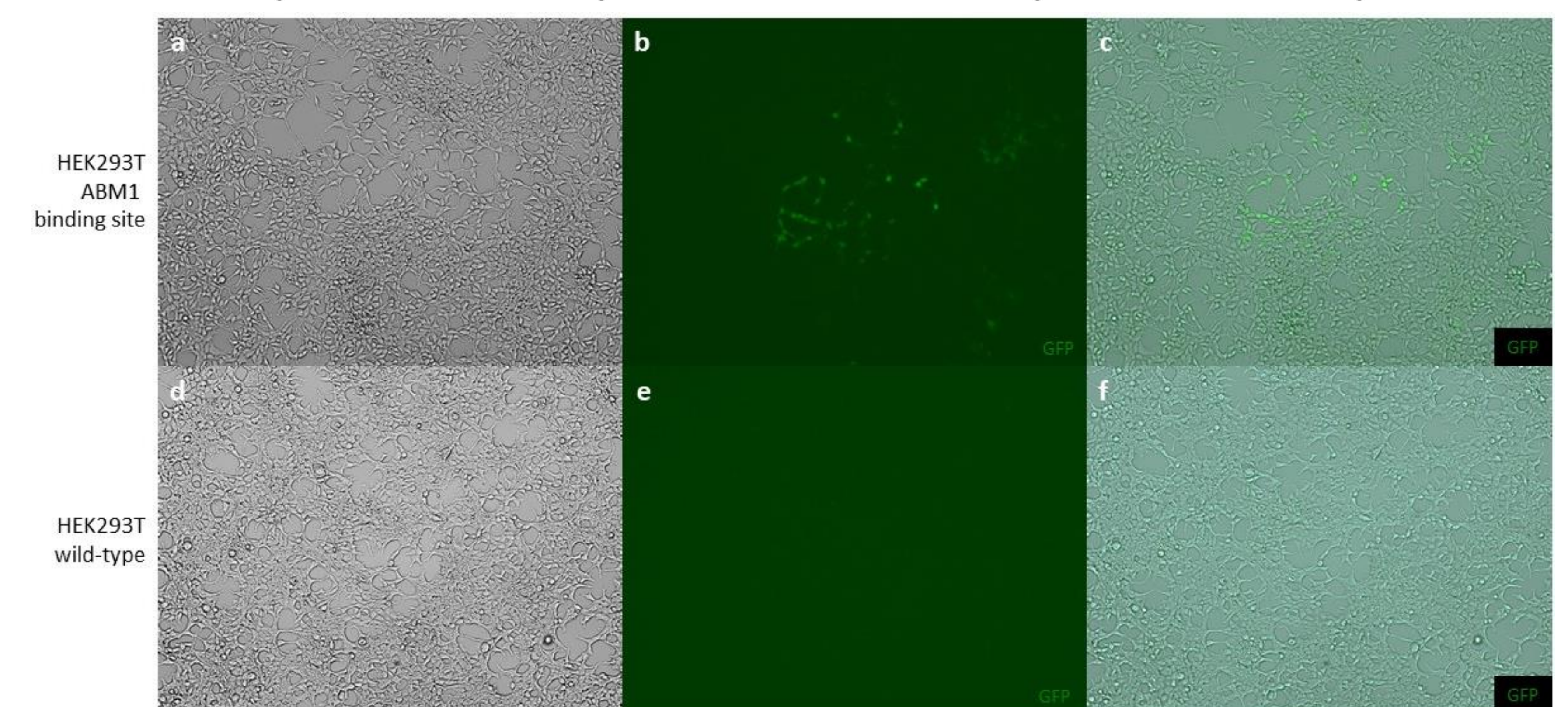

Figure 3. Results of fluorescent analysis of cells transduced with viral vector. Green signal stands for the fluorescent protein encoded by the introduced vector.

• CONCLUSIONS

Obtained results suggest that designed system seems to be **a promissing method** to control an expression of **two different transgenes** using non-xenobiotic drugs. According to luminescence leves observed in this study it is possible to modulate the expression of each transgene separately but also by means of another set of chemical compounds it is possible to silence or enhance the expression of both transgenes at the same time.
It is necessary to obtain cells with viral vector encoding red and green fluorescent protein to ensure that observations under fluorescent microscope can be performed in order that the expression regulation of two transgenes in same cell is feasible.
Also more specific ABM1 agonist and antagonist have to be found.

• REFERENCES

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• Scientific achievements and other activity directly related to implementation of doctoral dissertation:

Publication of the scientific paper:
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