989 A LUCIFERASE-BASED METHOD TO ASSESS ANTIGEN-SPECIFIC T CELL RESPONSES AND ANTIGEN PRESENTATION TO EVALUATE IMMUNOMODULATORY CHECKPOINTS AND THERAPEUTICS

Jimena Alvarez Freile*, Yuzhu Qi, Lisa Jacob, Maria Franceskin Lobo, Harm Jan Lourens, Gerwin Huls, Edwin Bremer. *Universitair Medical Centrum Groningen (UMCG), Groningen, Netherlands*

Background Targeted reactivation of the immune system, e.g. using immune checkpoint inhibitors (ICI), has shown great potential in the treatment of cancer. However, *in vitro* tools to rapidly investigate the impact of checkpoints in the context of specific T cell receptor (TCR) activation as well therapeutic effects of ICI treatment are lacking. Here, we have developed a simple method using the human papilloma virus 16 (HPV-16) E7-peptide (HLA-A2*02:01) and corresponding antigen-specific NFAT-luciferase model to assess antigen presentation and antigen-specific T cell responses and evaluate the impact of immunosuppressive factors and therapeutics that target such factors.

Methods HPV-16_$E7_{11-20}$ peptide was used as model antigen.[1] Jurkat NFAT-luciferase cell line or isolated primary T cells were modified with a previously reported TCR recognizing the $E7_{11-20}$ peptide.[2] HLA-A2+/- cancer cells were pulsed and incubated with Jurkat/T cells, with and without the $E7_{11-20}$-TCR. To study E7 presentation endogenously, CaSki cells were used. For the luciferase-based assay, T cell activation was correlated with the luminescence read out. For primary T cells, CD25/CD69 expression (Flow Cytometry), cytokine production (ELISA) and clustering (microscopy) were evaluated. As proof of concept for monitoring immunomodulatory events, cells were also treated with TGF-beta or modified to overexpress inhibitory immune checkpoints (e.g., HLA-G, VISTA).

Results Upon $E7_{11-20}$ pulsing of HLA-A2+ cells, an E:T ratio dependent increase in luminescence compared to non-pulsed cells (2-25 fold-increase) was observed upon coculture with Jurkat $E7_{11-20}$-TCR but not with parental Jurkat cells. Analogous experiments with CaSki yielded 30% increase in luminescence, demonstrating that the method is valid for measuring endogenous $E7_{11-20}$ processing and MHC presentation. Both HLA-A2+ pulsed cells and CaSki triggered specific T cell activation, illustrated by 5-20 fold-increase in MFI values for CD25 and CD69 expression and T cell clustering. For cytokine production, only the combination peptide: $E7_{11-20}$-TCR significantly triggered TNFa and IFNy production in HLA-A2 + pulsed cells, whereas for CaSki only higher IFNy production was observed. Overexpression of inhibitory molecules, such as HLA-G and VISTA, had a significant negative impact on TCR-induced luminescence compared to EV or non-treated cells. Moreover, treatment with immunosuppressive cytokines such as TGF-beta significantly impacted on luminescence.

Conclusions The MHC-I-specific Jurkat $E7_{11-20}$-TCR NFAT-luciferase system presented here can be used as an alternative method for the rapid evaluation of antigen-specific T cell responses *in vitro*. This method may be used as a rapid tool to study the impact of the TME or novel ICI in triggering effective T cell responses within the immune-oncology field.

REFERENCES

1. Riemer AB, *et al*. A conserved E7-derived cytotoxic T lymphocyte epitope expressed on human papillomavirus 16-transformed HLA-A2+ epithelial cancers. *J Biol Chem*. 2010;**285**(38):29608–29622.
2. Jin BY, Campbell TE, Draper LM, Stevanovic S, Weissbrich B Yu, *et al*. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. *JCI Insight* 2018. pmid:29669936

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