SCIENTIFIC REPORTS

OPEN

# An oligo-His-tag of a targeting module does not influence its biodistribution and the retargeting capabilities of UniCAR T cells

Received: 24 January 2019
Accepted: 18 April 2019
Published online: 22 July 2019

Justyna Jureczek[1,2], Ralf Bergmann[3], Nicole Berndt[1,3], Stefanie Koristka[3], Alexandra Kegler[3], Edinson Puentes-Cala[4], Javier Andrés Soto[5], Claudia Arndt[3], Michael Bachmann[1,2,3,6] & Anja Feldmann[3]

**Recently, we established the controllable modular UniCAR platform technology to advance the efficacy and safety of CAR T cell therapy. The UniCAR system is composed of (i) target modules (TMs) and (ii) UniCAR armed T cells. TMs are bispecific molecules that are able to bind to the tumor cell surface and simultaneously to UniCAR T cells. For interaction with UniCAR T cells, TMs contain a peptide epitope sequence which is recognised by UniCAR T cells. So far, a series of TMs against a variety of tumor targets including against the prostate stem cell antigen (PSCA) were constructed and functionally characterised. In order to facilitate their purification all these TMs are expressed as recombinant proteins equipped with an oligo-His-tag. The aim of the here presented manuscript was to learn whether or not the oligo-His-tag of the TM influences the UniCAR system. For this purpose, we constructed TMs against PSCA equipped with or lacking an oligo-His-tag. Both TMs were compared side by side including for functionality and biodistribution. According to our data, an oligo-His-tag of a UniCAR TM has only little if any effect on its binding affinity, *in vitro* and *in vivo* killing capability and *in vivo* biodistribution.**

Chimeric antigen receptors (CARs) are synthetic receptors comprising an antigen recognition domain, mostly fused to the signalling domain of the CD3ζ chain with one or more co-stimulatory domain(s)[1–4]. CAR engineered immune cells can target surface antigens independently of MHC expression. While impressive clinical responses were reported in patients with hematological malignancies[5–15], several obstacles prevent the broader application of the CAR technology especially for solid tumors. For instance, the expression of most if not all tumor-associated antigens (TAAs) is not limited to tumor cells. Varying levels of TAAs are also found on non-malignant cells of vital tissues. In order to increase the safety of the CAR technology and to minimise on-target/off-tumor toxicities but also other potentially life-threatening side effects, such as tumor lysis syndrome and cytokine release syndrome, a series of strategies have been developed including for example the use of suicide genes, the CRISPR/Cas9 system, targeting of co-expressed surface antigens, or gated targeting strategies[16–21].

An alternative way to control the activity of artificial receptors is the imitation of natural ligand/receptor systems. Already in 2012, Urbanska *et al.* described such an artificial receptor system based on chicken avidin as artificial extracellular receptor domain instead of an anti-TAA antibody domain of a CAR[22]. T cells modified with such artificial avidin receptors are inactive but can interact with target cells via biotinylated adaptor molecules e.g. biotinylated antibodies[22,23]. However, the antigenicity of chicken avidin or bacterial streptavidin and the presence of natural anti-biotin antibodies in sera of healthy individuals[24] might limit the use of such receptors in humans. To overcome such limitations we described in 2014 a modular CAR system termed UniCAR system[25]. Since then

[1]German Cancer Consortium (DKTK), partner site Dresden and German Cancer Research Center (DKFZ), Heidelberg, Germany. [2]University Cancer Center (UCC), Tumor Immunology, University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany. [3]Institute of Radiopharmaceutical Cancer Research, Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Dresden, Germany. [4]Corporación para la Investigación de la Corrosión (CIC), Piedecuesta, Colombia. [5]Universidad de Santander, Cúcuta, Colombia. [6]National Center for Tumor Diseases (NCT), University Hospital Carl Gustav Carus, TU Dresden, Dresden, Germany. Correspondence and requests for materials should be addressed to M.B. (email: m.bachmann@hzdr.de)