European Funds Smart Growth

HELIX IMMUNO ONCOLOGY

HELIXBIOPHARMA

European Union
European Regional Development Fund

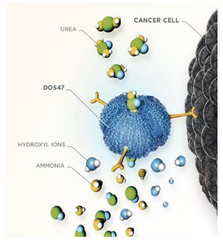
Targeting urease to human VEGFR2 elicits antitumor activity in triple-negative breast cancer models

Angelika Muchowicz[1], Anna Bujak[1], Beata Pyrzynska[1], Justyna Karolczak[1], Abdessamad Zerrouqi[1], Magdalena Ozga[1], Łukasz Szewczyk[1], Baomin Tian[2], Wah Wong[2], Marni Uger[2], Katarzyna Poplawska[1], Dorota Gierej[1], Pawel Wisniewski[1], Heman Chao[2], Magdalena Winiarska[1], Radoslaw Zagozdzon[1]
[1] Helix Immuno-Oncology, Warsaw, Poland; [2] Helix BioPharma Corp., Ontario, Canada
Company contact details: Paulina Koza, PhD p.koza@heliximmuno.com

Introduction

Angiogenesis is the process of new blood vessel formation and is essential for a tumor to grow beyond a certain size. Tumors secrete the pro-angiogenic vascular endothelial growth factor (VEGF), which acts upon local endothelial cells by binding to vascular endothelial growth factor receptors (VEGFR). Of the three VEGFR receptors, VEGFR2 expression is one of the most prominent biomarkers of the tumor-associated neovasculature regulating endothelial cell proliferation and migration. Moreover, VEGFR2 can be aberrantly expressed on the surface of tumor cells.

We have developed of V21-DOS47, an immunoconjugate composed of the VHH-portion of a camelid single domain anti-VEGFR2 antibody (V21H4) and jack bean urease, which converts endogenous urea into ammonia and hydroxyl ions.



V21-DOS47 is the second in a class of antibody-urease drugs. The first, L-DOS47, is currently in clinical trials for non-small cell lung cancer.

In this study, we identified tumor cells with VEGFR2 expression, tested V21-DOS47 binding to hVEGFR2 overexpressing cells, and investigated in vivo activity in both immunocompetent and immunodeficient mice.

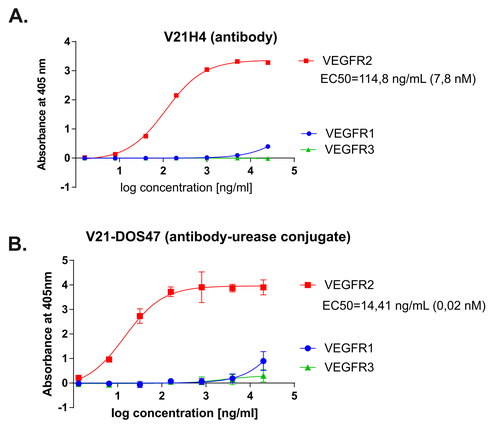
Methods

Flow cytometry experiments were performed with biotinylated V21H4 antibody and anti-biotin PE-conjugated secondary antibody. Detection of urease by flow cytometry was performed with an anti-urease antibody followed by incubation with a PE-conjugated secondary antibody. Western blotting was used for the assessment of protein expression and phosphorylation status of VEGFR2. For in vivo experiments, VEGFR2-overexpressing derivatives of human MDA-MB-231 and murine 4T1 breast cancer cell lines were generated. Balb/c or nude mice were inoculated with tumor cells on day 0 of each experiment. Intravenous injections of V21-DOS47 at a dose of 10 µg/kg were started on day 3 and continued on days: 5, 7, 9 and 11.

Conclusions

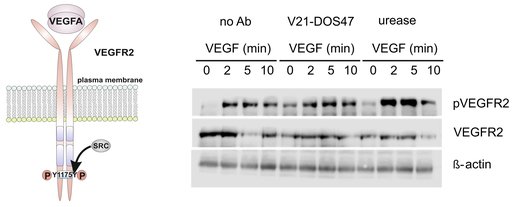
In summary, our data show successful targeting of the DOS47 platform to human VEGFR2 expressed on tumor cells. Our in vivo data indicate that the antitumor activity of V21-DOS47 is enhanced in immunocompetent mice, which suggests that the immune system is a significant component of the antitumor activity of the V21-DOS47 immunoconjugate.

V21H4 and V21-DOS47 bind hVEGFR2 antigen



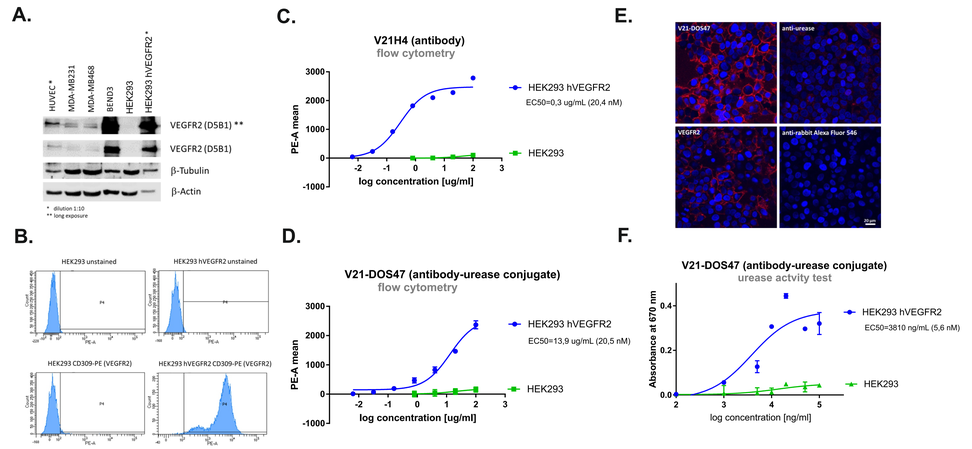
V21H4 antibody was biotinylated or conjugated to urease. Binding to immobilized recombinant VEGFR2/Fc was determined with ELISA assays with detection of biotinylated **(A)** or urease-conjugated **(B)** V21H4 antibody over a wide concentration range.

Effect of V21-DOS47 on VEGFR2 signaling



Phosphorylation of VEGFR2 (Y 1175) was checked by Western blotting in HEK293 hVEGFR2 cells upon incubation with VEGF and V21-DOS47 (5ug/ml).

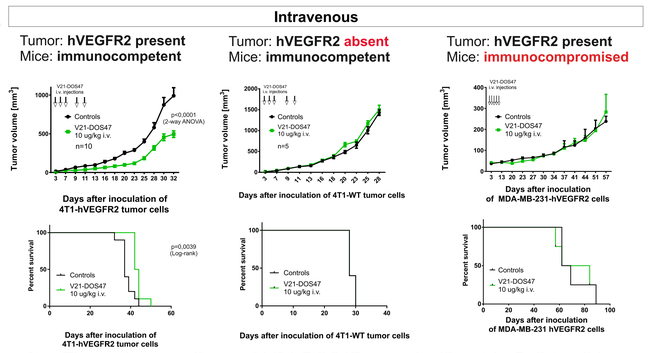
V21H4 and V21-DOS47 bind human VEGFR2



HEK293 cells were modified to express human VEGFR2 and verified with Western blot, in comparison to other tumor (MDA-MB-231 and 468) and endothelial (HUVEC, BEND3) cell lines **(A)**, and with flow cytometry **(B).** Binding of biotinylated V21H4 antibody **(C)** or V21-DOS47 **(D)** was determined with flow cytometry over a wide concentration range. Confocal microscopy was used to determine the localization of VEGFR2 with commercial antibody and to detect binding of V21-DOS47 to HEK293 hVEGFR2 cells **(E)**. Activity of urease conjugated to V21-DOS47 was determined with urease activity test and its ability to generate ammonia **(F).**

Antitumor effects of V21-DOS47 in vivo

Balb/c-wild-type or Balb/c-nude mice were inoculated with tumor cells: 2.5 x 10(5) 4T1 (exogenously overexpressing VEGFR2 or wild-type) or 1x10(6) MDA-MB-231-hVEGFR2, respectively, on Day 0 of each experiment. **Intravenous** (**Panels A**) or **intratumoral** (**Panel B**) injections of V21-DOS47 at dose of 10 µg/kg were started on Day 3 and continued on Days: 5, 7, 9 and 11. Tumors were measured with calipers and „survival" refers to reaching the humane endpoint.



Results: The antitumor effects of V21-DOS47 was significant in Balb/c-wild-type mice implanted with with 4T1-hVEGFR2 cells, but not with 4T1-wild-type cells or in Balb/c-nude mice implanted with MDA-MB-231-hVEGFR2 tumor cells. Such phenomenon suggests dependence of V21-DOS47-mediated antitumor actions on the eliciting the immune response.

Results: The most pronounced antitumor effects, both on tumor growth and mouse survival, of V21-DOS47 in Balb/c-wild-type mice implanted with 4T1-hVEGFR2 cells were observed when the conjugate was injected intratumorally.

References

Production and Characterization of a Camelid Single Domain Antibody–Urease Enzyme Conjugate for the Treatment of Cancer, Baomin Tian, Wah Yau Wong, Elda Hegmann, Kim Gaspar, Praveen Kumar, and Heman Chao; Bioconjugate Chemistry 2015 26 (6),1144-1155
DOI: 10.1021/acs.bioconjchem.5b00237

„Novel, efficient, potentially nontoxic and highly specific breast cancer therapy development, based on patented DOS47 technological platform, enabling microenvironment and immune response modification by the change of pH", POIR.01.01.01-00-1645/15, NCBR