**POSTER PRESENTATION** 



**Open Access** 

## EGFRvIII TandAbs are specific and highly potent drug candidates for the treatment of solid tumors

Kristina Ellwanger<sup>1\*</sup>, Uwe Reusch<sup>1</sup>, Ivica Fucek<sup>1</sup>, Michael Weichel<sup>1</sup>, Thorsten Gantke<sup>1</sup>, Stefan Knackmuss<sup>1</sup>, Vera Molkenthin<sup>2</sup>, Jens-Peter Marschner<sup>1</sup>, Martin Treder<sup>1</sup>

*From* 30th Annual Meeting and Associated Programs of the Society for Immunotherapy of Cancer (SITC 2015) National Harbor, MD, USA. 4-8 November 2015

To harness the immune system's cytotoxic capacity to fight solid tumors, we developed tetravalent, bifunctional antibodies that recognize EGFRvIII, the deletion variant III of EGFR, and either CD3 or CD16A on immune cells, thereby directing T cells or NK-cells to eliminate EGFR-vIII<sup>+</sup> cancer cells.

Using phage display, we identified scFv antibodies that selectively bind to EGFRvIII. These highly EGFRvIIIspecific scFv antibodies were substantially improved by affinity maturation achieving K<sub>D</sub>s in the 100 pM range or lower and used to construct a set of bispecific EGFRvIII-targeting TandAbs with a broad range of binding and cytotoxic properties. Mono- and bivalent binding constants, specificity for EGFRvIII and CD3 or CD16A, cytotoxic activity, and target-dependent effector cell activation were characterized in a panel of in vitro assays. TandAbs exhibited exquisite specificity towards the EGFRvIII antigen in Western Blot, SPR, ELISA, and FACS assays of EGFRvIII<sup>+</sup> cells. No binding was observed to recombinant EGFR or to EGFR<sup>+</sup> cells. The TandAbs apparent affinities for EGFRvIII were up to 25-fold improved relative to the monovalently binding scFvs, resulting in a K<sub>D</sub> of 11 pM for the best TandAb.

EGFRvIII/CD3 and EGFRvIII/CD16A TandAbs with high affinity for EGFRvIII were similarly potent in killing assays, displaying cytotoxicity towards EGFRvIII<sup>+</sup> F98 glioma, transfected CHO or human DKMG cells with  $EC_{50}$  in the range of 1 pM – 10 pM. No cytotoxicity was observed on EGFR<sup>+</sup> cells or EGFRvIII-negative cells demonstrating the high selectivity of EGFRvIII TandAbs for the tumor-specific EGFRvIII. Importantly, in the absence of EGFRvIII<sup>+</sup> target cells *in vitro* TandAbs did not elicit T- or NK-cell activation, as demonstrated by their

<sup>1</sup>Affimed GmbH, Heidelberg, Germany

lack of proliferation. Binding to EGFRvIII in different solid tumor types and its absence from healthy tissues was shown by immunohistochemistry using a high affinity EGFRvIII-binding bivalent Diabody.

In summary, EGFRvIII/CD3 and EGFRvIII/CD16A TandAbs provide an opportunity to develop cytotoxic antibodies that solely target cancer, sparing normal tissues and thereby reduce the side effects associated with EGFR therapy.

## Authors' details

<sup>1</sup>Affimed GmbH, Heidelberg, Germany. <sup>2</sup>AbCheck s.r.o., Plzen, Czech Republic.

Published: 4 November 2015

doi:10.1186/2051-1426-3-S2-P219 Cite this article as: Ellwanger *et al.*: EGFRvIII TandAbs are specific and highly potent drug candidates for the treatment of solid tumors. *Journal for ImmunoTherapy of Cancer* 2015 3(Suppl 2):P219.

## Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

**BioMed** Central

Submit your manuscript at www.biomedcentral.com/submit



© 2015 Ellwanger et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http:// creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/ zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Full list of author information is available at the end of the article