T- or NK-cell recruiting anti-EGFRvIII TandAbs are highly specific and potent therapeutic antibody candidates for the treatment of EGFRvIII\(^+\) tumors

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Introduction

EGFRvIII is the most prevalent tumor-specific variant of the wild-type EGFR and represents an attractive target in various solid tumours such as GBM, HNSCC, NSCLC and others. Despite the high medical need and the tumor specificity of this target in these indications very little therapeutic progress has been made with EGFRvIII. Due to its exceptional tumor exclusivity it is an ideal candidate for both T- and NK-cell engagement. We therefore developed tetrameric, bispecific EGFRvIII antibodies (TandAbs) to recruit either cytotoxic T- or NK-cells, aimed at the elimination of EGFRvIII cancer cells while sparing normal tissue that ubiquitously expresses EGFR. Selected lead candidates AFM21 (EGFRvIII/CD16A) and AFM22 (EGFRvIII/CD16A4) exhibited exquisite specificity towards EGFRvIII in Western Blot, SPR, ELISA and flow cytometry. No binding was observed in EGFR. Both displayed potent cytotoxicity in the low picomolar range and, most importantly, did not elicit T- or NK-cell activation or activation of immune cell proliferation, thus suggesting an excellent safety profile.

In vivo efficacy for AFM21 and AFM22 mouse xenograft model. The clinical relevance of EGFRvIII as a tumor marker and the use of our anti-EGFRvIII domain as companion diagnostics were evaluated by IHC on GBM, HNSCC, NSCLC and others.

In summary, these in vitro and in vivo studies qualify AFM21 and AFM22 as highly attractive therapeutic antibody candidates and present themselves as promising strategies for selective immunotherapy for the treatment of EGFRvIII tumors. The highly specific tumor expression of EGFRvIII and the absence of off-target activity of our TandAbs provide for an excellent safety profile reducing the risks of undesired effects associated with other EGFR-driven therapies.

Highly specific binding

- Truncation of 4 N-terminal amines scales in the extracellular domain of EGFRvIII obscures binding of our EGFRvIII-specific antibodies
- Selective binding to the unique N-terminal EGFRvIII epitope was validated using suitable affinity enrichment strategies in Western Blot, ELISA and flow cytometry
- No binding to EGFR was observed with our antibodies

Protease mapping analysis: diferent pressure treatment conditions demonstrated binding to a N-terminal epitope resembling the EGFRvIII-specific novel glycon for our EGFRvIII specific antibody, whereas reference antibody LS4 showed a different pattern (data not shown).

AFM21 or AFM22 bind with high affinity and specificity to target and effector cells

AFM21: inhibition of tumor growth in vivo

AFM21 demonstrates anti-tumor efficacy and shows greater tumor growth inhibition than Celecoxib.

Summary/Conclusion

Fully characterized EGFRvIII-specific human scFv were formatted into TandAbs and AFM21 & AFM22 were selected as drug candidates

AFM21 & AFM22 show excellent productivity, purity and stability

Both drug candidates bind to the highly tumour-specific EGFRvIII epitope and this specificity was shown to be unique to our anti-EGFRvIII domains

AFM21 & AFM22 showed superior cytotoxicity towards various EGFRvIII-expressing cell lines to other competing antibodies, with EC50 values in the range of 1 to 10 pM, and no off-target activity

AFM21 mediates dose-dependent inhibition of EGFRvIII tumor growth in a mouse xenograft model

In conclusion, AFM21 and AFM22 are excellent and superior therapeutic agents when compared to other therapeutic competitors such as ABT-414 or a BITE-based approach against EGFRvIII expressing tumors