Highly cytotoxic EGFR/CD16A TandAbs specifically recruit NK cells to potently kill various types of solid tumors

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Introduction

Constitutive EGFR activation plays an important role in the pathophysiology of several solid cancers, such as colorectal, non small cell lung or squamous cell carcinomas of the head and neck. Tyrosine kinase inhibitors or monoclonal antibodies that prevent EGFR ligand binding, dimerization and activation have been approved for treatment of such cancers. However, despite demonstrated clinical efficacy, intrinsic or acquired resistance to such treatments has been described for a larger number of patients. Natural killer cells (NK-cells) play a central role in the innate immune system and have the capacity to destroy neoplastic cells. To specifically utilize the cytotoxic potential of NK-cells for the elimination of EGFR- cancer cells, tetranispic bispecific EGFR/CD16A NK-cell TandAbs, with two binding sites for CD16A expressed on tumor cells, and two binding sites for CD16A on NK-cells were developed.

Using antibody phage display, scFv recognizing novel epitopes in the extracellular domain of EGFR were identified and characterized. Bispecific EGFR/CD16A TandAbs were constructed and analyzed in terms of thermo-stability, binding and cytotoxicity. TandAbs containing our EGFR-specific domain were highly potent in cytotoxic assays towards EGFR- tumor cell lines or transformed CHO cells with single picomolar or subpicomolar EC₅₀ values. In contrast to NK-cell recruiting TandAbs containing the Fv sequences from cetuximab, TandAbs containing our EGFR-binding domain did not exhibit signs of thermal instability or aggregation. Our data suggest that EGFR/CD16A TandAbs are novel, highly potent drug candidates suitable for the treatment of EGFR expressing malignancies and overcoming intrinsic or acquired resistance to other drugs.

TandAbs are potent bispecific tetravalent antibodies

- **a. TandAb Features**
  - comprised of V, and V, domains
  - expressed as a single gene product
  - linkers of intermolecular head-to-tail homodimerization

- **b. TandAb Properties**
  - Blocked binding to antigens on target and on NK cells
  - No regulatory activity due to MW<100 kDa
  - Excellent drug-like properties (production and stability)

- **c. Mode of Action**
  - NK-cell recruitment (CD16A: NK-cell TandAbs Platform)

EGFR-specific Fv sequences were formatted into TandAbs

- Novel EGFR-specific scFvs were identified by phage display screening
- In addition, EGFR-binding Fv sequences of cetuximab (C225) were used to construct TandAbs as comparators
- All anti-EGFR domains were combined with previously described anti-CD16A domains to construct NK-cell recruiting TandAbs
- Various TandAbs differing in the order and position of two binding specificities (EGFR and CD16A) were generated and characterized

TandAbs with anti-EGFR in outer position

TandAbs with anti-EGFR in inner position

TandAbs generated with novel EGFR antibodies show excellent stability

- Incubation of purified TandAbs at 37°C or 4°C in buffer (50 mM) for 1, 3 and 7 days
- Analysis of molecular integrity and binding by size exclusion chromatography (SEC), high performance liquid chromatography (HPLC), mass spectrometry (MS), SEC-MALDI-TOF-MS
- SEC and MS analysis revealed that all purified TandAbs were stable at 37°C and 4°C for at least 7 days
- Clouding, aggregation, chain exchange, and high-molecular-weight species were not detected

TandAbs bind to human and cyno EGFR and CD16A

Binding of AFM4, T677 to CHO cells expressing human or cyno EGFR

AFM4 shows comparable binding to human and cyno EGFR

Highly cytotoxic binding of AFM4 enables efficient killing of EGFR^+ tumor cells and greater cytotoxicity than cetuximab

- **a. High affinity binding of AFM4 enables efficient killing of EGFR^+ tumor cells and greater cytotoxicity than cetuximab**
  - Colorectal-labeled A431 (A) or SK-MEL-2 (B) target cells were incubated with enriched human NK-cells at an E:T ratio of 1 in the presence of increasing antibody concentrations. After 4 h incubation the colonies formed from fixed target cells were quantified and used for the calculation of % specific lysis. Mean and SD of duplicates are plotted. EC₅₀ values were calculated by non-linear regression/logistic dose-response.

- **b. AFM4 demonstrates highly potent and efficacious tumor cell killing**
  - AFM4 shows superior tumor cell killing compared to cetuximab as shown by the lower EC₅₀ values (AFM4 cetuximab 1.5 pM). (Figure not shown)
  - NK-Cell recruitment results in superior anti-tumor activity of AFM4 in direct comparison to cetuximab

- **c. AFM4 shows excellent stability at 4°C, 37°C, and 3°C for up to 7 days in buffer and serum**
- **d. AFM4 shows comparable activity to EGFR of cyno (comparable potency) and human (higher potency) domains**

Summary

- **a. AFM4 shows excellent stability at 4°C, 37°C, and 3°C for up to 7 days in buffer and serum**
- **b. AFM4 binds specifically to human EGFR and to human CD16B, but not to human CD16B**
- **c. AFM4 shows comparable activity to EGFR of cyno (comparable potency) and human (higher potency) domains**
- **d. AFM4 shows superior killing of tumor cells in direct comparison to cetuximab**

Conclusion

- **a. The demonstrated clinical efficacy of anti-EGFR therapeutics, intrinsic or acquired resistance has been described for a substantial number of patients. We developed tetranispic bispecific EGFR/CD16A NK-cell recruiting TandAbs to engage the cytotoxic potential of NK-cells for the elimination of EGFR- expressing cancer cells.**
- The combination of high affinity EGFR binding domains with potent NK-cell recruitment is hypothecated to overcome such therapeutic resistance and lead to improved tumor cell killing compared to EGFR-targeting IgGs such as cetuximab, or small molecule inhibitors such as erlotinib.
- NK-cells play a central role in the innate immune system and have the capacity to not only destroy neoplastic cells, but also to shape the tumor microenvironment as previously shown for AFM131 in vivo. Furthermore, combinations with check-point modulators may augment anti-tumor efficacy in solid tumors with high medical need.

Taken together, our data suggest that AFM4 is a novel, highly potent drug candidate suitable for the treatment of EGFR-expressing malignancies.