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

Introduction

To harness the cytotoxic capacity of immune cells for the treatment of several types of solid tumors, we developed tetravalent, bifunctional antibodies that recognize EGFRvIII, the deletion variant III of EGFR, and CD3 or CD16A on immune cells, thereby directing T-cells or NK-cells to eliminate EGFRvIII+ cancer cells. The tumor-restricted expression of EGFRvIII suggested by IHC provides an opportunity to solely target cancer, spare normal tissues and thereby reduce the side effects associated with EGFR therapy. Using phase display, we identified scFv antibodies that selectively bind to EGFRvIII. These highly EGFRvIII-specific scFvs were substantially improved by affinity maturation achieving Kd's in the 100 pM range or lower and were used to construct a set of bispecific EGFRvIII-targeting TandAbs with a broad range of binding and cytotoxic properties. EGFRvIII-targeting TandAbs exhibited exquisite specificity towards EGFRvIII antigen in Western Blot, SPR, ELISA, and FACs assays of EGFRvIII+ cells. EGFRvIII+CD3 TandAbs with high affinities were most potent in killing assays, displaying cytotoxicity towards EGFRvIII+ F98 glioma, CHO or human DMKG cells with EC50 in the range of 1-10 pM. Biophysical, functional and pharmacological characterization of several candidates is still ongoing, whereby a first non-optimized EGFRvIII+CD3 TandAbs demonstrated a robust dose-dependent growth retardation in a proof-of-concept EGFRvIII+ subcutaneous xenograft tumor model.

TandAbs are potent bispecific tetravalent antibodies

- TandAbs consist of scFv Vα and V domains
- Expressed as a single protein
- Links T via immuno-intermediate head-to-tail interactions

TandAb Properties

- Bivalent binding of antigens on target and effector cells
- Potent cytotoxicity against target cells
- No Fo-associate side effects
- Favorable half-life due to MW >100 kDa
- Excellent drug-like properties (production and stability)

Mode of Action

- T-cell recruitment (CD3 T-cell TandAbs PLATFORM)

EGFRvIII is associated with oncogenic transformation

- Most frequent mutant variant of EGFR
- In-frame deletion of exons 2-7
- Truncation of 2/3 of EGFR
- Novel glycine residue at the junction
- Highly tumor-specific epitope
- Suggesting the potential for CD3 induction and activation
- Contributing enhanced tumorigenesis and resistance to conventional anti-cancer therapy

EGFRvIII specific scFv were isolated by phage display and formatted into TandAbs

- TandAb variants differing in the domain order were constructed, expressed and characterized
- Effector domain binding to only human (CD3) or syngeneic and human CD5 (CD16A) or CD14 were used in TandAbs

EGFRvIII targeting TandAbs are well expressed and stable

- EGFRvIII-targeting TandAbs are well expressed with research stage expression levels in the range of or higher than a clinical stage TcCell recruiting TandAbs with validated manufacturability
- EGFRvIII-targeting TandAbs show good thermostability and other drug-like properties

TandAb gene expression

- Anti-EGFRvIII Diabody specifically and exclusively stains cancer tissues in IHC
- EGFRvIII is prevalent in several solid tumor types but not expressed in healthy tissues

Proof-of-concept in vivo: Dose-dependent inhibition of tumor growth

- EGFRvIII+CD3 TandAbs-mediated tumor growth inhibition is dose-dependent and pronounced in xenograft models
- EGFRvIII-targeting TandAbs exhibited robust dose-dependent growth retardation in a proof-of-concept EGFRvIII+ subcutaneous xenograft tumor model.

EGFRvIII specific Affinity maturation using Abacell library screening and re-formatting into TandAbs

- 5 affinity matured scFv were selected for the construction of new TandAbs
- Binding selectivity was maintained by ELISA and on EGFRvIII or EGFRvIII CHO or F98 cells (by FACS)
- Improved EGFRvIII binding Kd in TandAbs is due to reduced k

High affinity binding of EGFRvIII+CD3 TandAbs enables efficient killing of EGFRvIII+ cells

- EGFRvIII+CD3 TandAbs containing affinity matured EGFRvIII binding domains show significantly enhanced retention on the cell surface of EGFRvIII+ cells
- EGFRvIII+CD3 TandAbs are highly efficacious, potent and specific in inducing T-cell-mediated killing of EGFRvIII+ target cells

No off-target activity of EGFRvIII+CD3 TandAbs

- No off-target activation of EGFRvIII+CD3 TandAbs: No TandAbs are able to interact with T and B cells at concentrations up to 30 µg/ml

Summary/Conclusion

- EGFRvIII-specific human scFv isolated and affinity matured to picomolar Kd
- EGFRvIII+CD3 TandAbs variants with excellent productivity, purity and stability
- Highly potent and efficacious TandAbs were generated and characterized
- EGFRvIII+CD3 TandAbs are highly efficacious, potent and specific in inducing T-cell-mediated killing of EGFRvIII+ target cells
- Affinity improved TandAbs display cytotoxicity towards EGFRvIII-expressing CHO, CD3 or DMKG cells with EC50 in the range of 1-10 pM.
- EGFRvIII+CD3 TandAbs exhibit exquisite binding specificity towards EGFRvIII+ with no binding to the wildtype EGFR and are highly selective in mediating lys of EGFRvIII+ targets without any off-target activity
- EGFRvIII+CD3 TandAbs mediate dose-dependent inhibition of EGFRvIII+ tumor growth in a mouse xenograft model
- Affinity optimized EGFRvIII+CD3 TandAbs represent novel highly potent drug candidates for the treatment of EGFRvIII+ malignancies

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