Human Anti-Human Epidermal Growth Factor Receptor Polyclonal Antibodies Block Ligand Binding and Increase Survival of Non-small Cell Lung Carcinoma Xenograft Mice

Daniel W. Vermeer, Paul L. Colbert, Tom C. Luke, Hua Wu, Christoph L. Bausch and Kristi A. Egland
SAB Biotherapeutics, Sioux Falls, SD

Abstract

Human polyclonal antibodies (pAbs) generated from the Diversitab™ transchromosomic bovine platform are being evaluated in preclinical studies and human clinical trials for infectious disease, and autoimmune disorders, including Type 1 Diabetes indications. Using this platform technology, specific tumor targeted pAbs to treat human cancers are also under development. The Epithelial Growth Factor Receptor (EGFR) mediates downstream cell signaling pathways that control fundamental cellular functions. Although several anti-EGFR monoclonal antibodies, such as cetuximab, are approved for clinical use to treat EGFR-positive non-small cell lung cancer (NSCLC), colorectal cancer, and head and neck cancer. Tumors which initially respond to these monoclonal antibodies eventually become resistant. Because better immunotherapies are needed, we produced a human anti-EGFR pAb (SAB-162E) and herein report pre-clinical *in vitro* and *in vivo* results. Consistent with the ability of pAbs to bind multiple epitopes on protein targets, SAB-162E bound EGFR at higher levels than cetuximab as measured by both ELISA analysis with recombinant proteins and a cell surface binding assay. Binding to the EGFR truncation mutant, EGFRvIII, was also analyzed by ELISA, and SAB-162E had a higher level of binding to EGFRvIII compared to cetuximab. SAB-162E demonstrated complete blockade of EGF ligand binding to EGFR-positive cells and altered EGFR downstream signaling pathways by decreasing the phosphorylation status of Erk1/2, Akt and p38. Functionally, this signaling blockade led to a decrease in cellular proliferation. SAB-162E decreased *in vitro* cellular migration of a human NSCLC cell line containing a wild-type EGFR signaling pathway. Additionally, SAB-162E effector functions were investigated including complement dependent cytotoxicity (CDC) and antibody dependent cellular cytotoxicity (ADCC). SAB-162E activated CDC on *EGFR*-expressing cells, while cetuximab did not. To measure ADCC activity, a reporter assay with FcγRIIIa Jurkat effector cells was used and demonstrated that SAB-162E initiated ADCC activity. Lastly, using a human NSCLC cell line-derived xenograft (CDX) model in immunodeficient mice, treatment with SAB-162E decreased tumor growth and almost tripled survival time. These findings provide evidence that SAB-162E represents a promising first-in-class human pAb therapeutic for EGFR-positive cancers and the potential use of the Diversitab™ platform to produce oncology therapeutics.

Background

1. DiversitAb™ Development Process for generation of human anti-human EGFR polyclonal antibodies, SAB-162E.

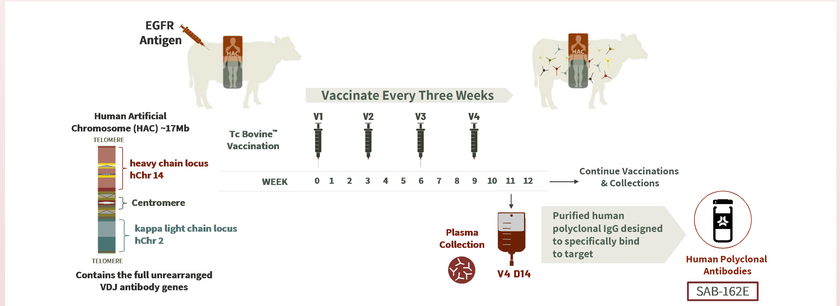


Figure 1. Generation of human anti-human EGFR polyclonal antibodies (SAB-162E) using the DiversitAb™ platform.
A single Tc Bovine was immunized with 2 mg of EGFR-hFc formulated with adjuvant for vaccinations V1 and V2. For V3 and V4, 5 mg of EGFR-hFc plus adjuvant was administered per vaccination for a total of four vaccinations via intramuscular injections. Serum was collected from the Tc bovine over the course of the four immunizations. Plasma was collected 14-days after the fourth vaccination (V4D14), and the human IgG fraction was purified by caprylic acid fractionation and column chromatography. This drug product is referred to as SAB-162E.

2. SAB-162E binds to recombinant and cell surface EGFR.

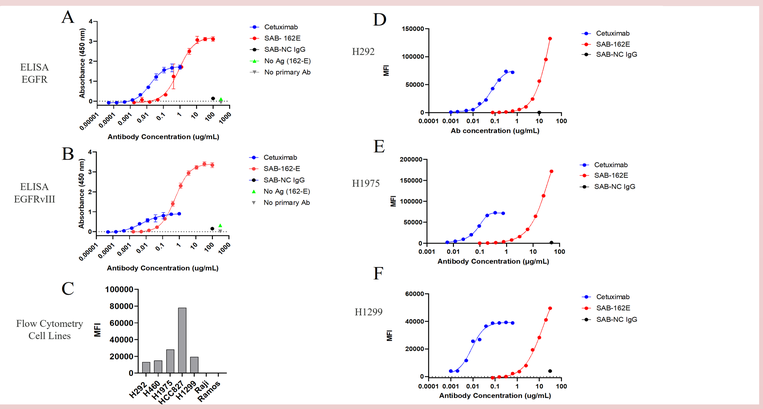


Figure 2. SAB-162E binding to EGFR and EGFRvIII.
Binding of SAB-162E to EGFR protein (A) or EGFRvIII (B) by indirect ELISA is depicted as absorbance on the y-axis plotted against antibody concentration on the x-axis. Data points are means of technical triplicates with error bars depicting ±SD. One representative experiment of three biological replicates is shown. (C) Flow cytometry analysis of cellular SAB-162E binding to known EGFR positive NSCLC cells lines H292, H1975 and H1299, as well as EGFR negative Raji and Ramos cell lines. Bars represent mean fluorescence intensity (MFI) of ≥3,000 singlet live cells on the y-axis. Cell lines are indicated on the x-axis. Representative data from one biological replicate of triplicate experiments is shown. (D-F) Flow cytometry titrations of antibody binding to cellular EGFR on three NSCLC cell lines (D) H292, (E) H1975 and (F) H1299 are shown. Data points at each antibody dilution represent MFI for ≥3,000 singlet live cells. Representative data from one biological replicate of triplicate experiments is shown.

Results

3. SAB-162E blocks EGF- EGFR binding and downstream signaling.

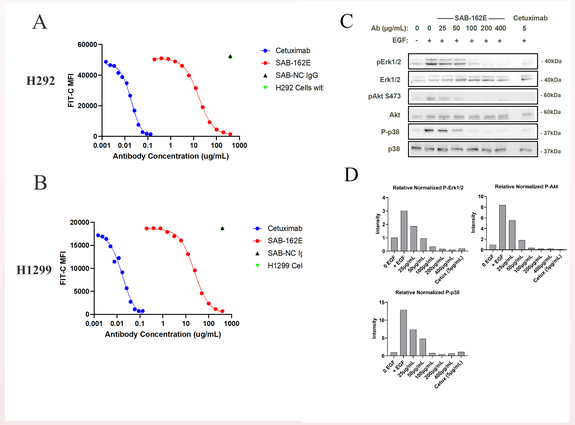


Figure 3. SAB-162E blockade of EGF- EGFR binding and ensuing downstream signaling.
SAB162E blockade of saturating EGF-Alexa Fluor binding is shown by MFI on the y-axis plotted against serial antibody dilutions on the x-axis for EGFR-positive human NSCLC cell lines (A) H292 and (B) H1299. Data points are MFI from ≥3,000 singlet live cells. Representative data from one replicate of biological triplicate experiments is shown for each NSCLC cell line. (C) SAB-162E ligand blockade of downstream signaling pathways in H292 cells shown by Western immunoblot analysis with increasing levels of SAB-162E for both the total and phosphorylated proteins Erk, Akt and p38. (D) Phosphorylation normalized to total protein and expressed relative to unstimulated cells determined by densitometry and graphed at each SAB-162E concentration.

4. SAB-162E decreases cellular proliferation.

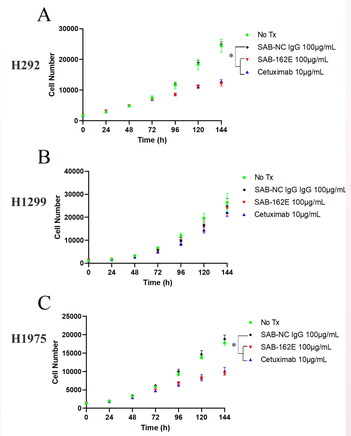


Figure 3

Figure 4. Effect of SAB-162E on cellular proliferation.
Growth curves of NSCLC cells treated with negative control pAb (SAB-NC IgG), SAB-162E, or cetuximab. Viable cell counts (y-axis) are plotted against time (x-axis) for (A) H292 cells lacking downstream pathway activating mutations, (B) H1299 cells with downstream pathway mutations or (C) H1975 cells with EGFR activating mutations. Each data point represents five technical replicates, error bars are SD. Representative data from one biological replicate of triplicate experiments is shown. Analysis by 2-way repeated measures ANOVA. n.s. not significant, * p≤0.05, **p≤0.005.

5. SAB-162E inhibits cellular migration of NSCLC cells.

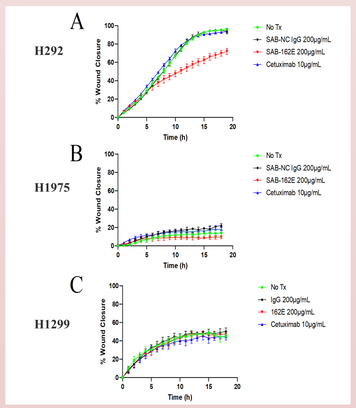
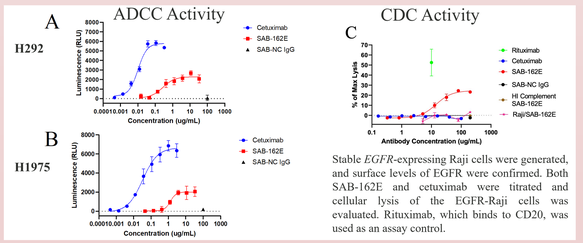


Figure 5. Effect of SAB-162E on cellular motility.
Inhibition of migratory capacity by the indicated treatment is shown as percent wound closure on the y-axis plotted against time on the x-axis for (A) H292 (B) H1975 and (C) H1299 NSCLC cells. Each data point represents five technical replicates with error bars shown as ±SD. Representative data from one biological replicate of triplicate experiments is shown. Analysis by 2-way repeated measures ANOVA. n.s. not significant, * p≤0.05, **p≤0.005

6. SAB-162E activates ADCC and CDC effector functions.



Stable *EGFR*-expressing Raji cells were generated, and surface levels of EGFR were confirmed. Both SAB-162E and cetuximab were titrated and cellular lysis of the EGFR-Raji cells was evaluated. Rituximab, which binds to CD20, was used as an assay control.

Figure 6. Activation of ADCC and CDC by SAB-162E.
ADCC assays against target NSCLC cell lines (A) H292 and (B) H1975 were performed with a 6:1 E:T cell ratio and measured after 24 or 48 hours as indicated on the graph. Activation of ADCC effector cells is depicted by luciferase activity in RLU on the y-axis verses increasing antibody concentration on the x-axis for each of the indicated treatments. The nonspecific pAb control, SAB-NC IgG, is shown only at the highest antibody concentration. Data points are means of technical triplicates. Error bars indicate ±SD. Representative data from one replicate of biological triplicate experiments are shown. (C) Complement dependent cell lysis is shown as a percentage of maximum lysis on the y-axis calculated from untreated detergent lysed wells 15 minutes after complement addition. The single Rituximab concentration serves as a positive assay control and heat inactivated complement samples demonstrate complement dependent lysis, while un-transduced Raji cells and SAB-NC IgG demonstrate antigen specificity. Data points are represented as means of technical triplicates ±SD and experiments were repeated three times with data from one representative experiment shown.

Acknowledgements

A kind gift from Christine and Eddie Hamilton supported this research.

7. SAB-162E decreased mouse *in vivo* tumor growth and almost tripled survival time.

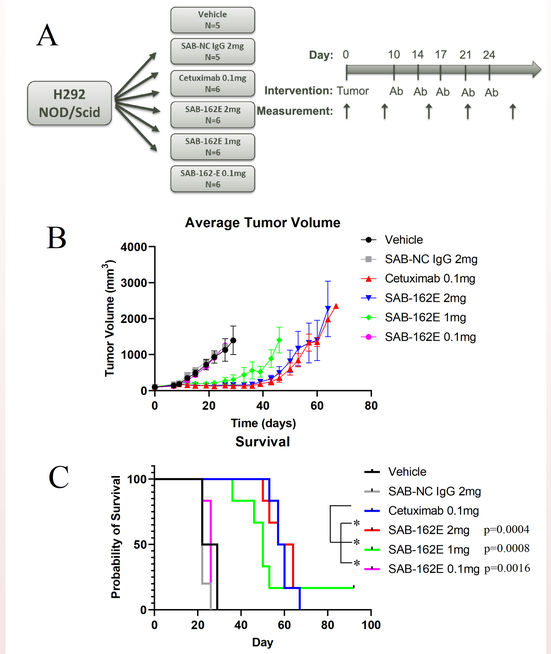


Figure 7. SAB-162E *in vivo* efficacy dose response study.
H292 NSCLC cells injected into the hind limb of NOD/SCID mice were allowed to establish for 10 days at which time animals were treated with the indicated dose of SAB-162E, cetuximab, antibody diluent, or SAB-NC IgG as represented in the schematic (A). Investigators were blinded to both treatment modality and group throughout the study. Tumor growth was followed by caliper measurement, and average tumor volume curves are plotted with data points represented as means and error bars ±SEM (B). At study completion, survival was analyzed by Kaplan Meir survival analysis. Death events due to predefined volumes or tumor related morbidities were recorded (C). n.s. not significant, * p≤0.05, **p≤0.005

Conclusions

- The DiversitAb platform was utilized to generate human pAbs against EGFR, referred to as SAB-162E.
- SAB-162E binds to EGFR-positive cells in a dose dependent manner and can reach a higher level of overall binding than a monoclonal antibody.
- Data suggests that SAB-162E binds to multiple epitopes on EGFR and truncated EGFRvIII compared to cetuximab.
- Binding of the EGF ligand to EGFR on NSCLC cells was blocked by SAB-162E.
- In NSCLC cells, SAB-162E reduced downstream phosphorylation of EGFR signal transduction proteins and decreased cellular proliferation.
- Target specific ADCC activation was demonstrated by SAB-162E in NSCLC cell lines.
- Complement-dependent cell lysis was induced by SAB-162E in a dose dependent manner.
- Treatment of a NOD/SCID mouse CDX H292 model with SAB-162E indicated a dose responsive effect on tumor volume. The highest dose significantly slowed tumor growth and tripled survival time.
- First-in-class human pAb therapeutics could have distinct advantages over monoclonal antibodies, such as targeting cancer types with high mutational burden or cancers with multiple targets.
- The DiversitAb platform is a powerful tool to generate targeted human pAbs against a variety of tumor-associated antigens, such as CD20, EGFRvIII and Her-2, for the treatment of complex human cancers.