PA-039

Differential impact of BCMA (*TNFRSF17*) extracellular domain mutations on *in vitro* potency of elranatamab versus teclistamab

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Objectives

Here, we performed *in vitro* studies to compare the impact of four BCMA mutations (BCMAmuts), recently identified in multiple myeloma (MM) patients relapsing on BCMA-directed T cell engager (TCE) therapy[1], on elranatamab (elra) and teclistamab (tec) binding and tumor killing potency.

Conclusions

Our *in vitro* results suggest BCMAmuts may differentially impact BCMAxCD3 TCE activity, although the clinical relevance remains uncertain. Not all mutations may impact TCE potency, such as P33S (germline). Preclinically, elra T cell activation and killing potency was similar to or higher than tec. As preclinical binding affinity and killing potency is maintained, elra may have increased activity for patients harboring the S30del mutation.

Further understanding mutation prevalence may inform if various BCMAxCD3 TCEs select for specific mutations, which could inform optimal treatment sequencing and selection, although these results must be clinically validated.

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References: **1**. Lee H, et al. Nature Medicine 2023;**29**:2295-2306.

Disclosures: MJ, RS, TC, CL, and KBM are current employees of Pfizer, Inc. LM is a former employee of Pfizer, Inc. MJ, CL, LM, and KBM hold Pfizer stock.

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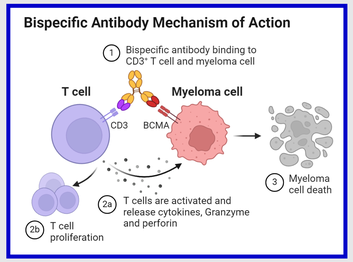
Background

BCMA (B cell maturation antigen) is a surface protein expressed by malignant plasma cells in multiple myeloma (MM). Elranatamab (elra) and teclistamab (tec) are bispecific BCMAxCD3 T cell engagers (TCEs) commercially approved for treating relapsed/refractory MM (RRMM) patients. BCMAxCD3 TCEs mediate tumor killing by binding to T cells via CD3 and tumor cells via BCMA.

BCMA mutations (BCMAmuts) may represent potential resistance mechanisms in some RRMM patients progressing on BCMA TCEs. In a small cohort of such patients (n=11), R27P, P33S, and P34del mutations were identified in patients progressing on elra, and S30del and P34del mutations were identified in patients progressing on tec[1]. R27P, S30del, and P34del were reported to decrease the potency of select BCMA TCEs in vitro[1].



Methods

AlphaFold modeling was performed to predict elra's parental anti-BCMA Fab binding to BCMAmuts.

For all *in vitro* work, commercial grade elra and tec were used. Binding affinity to recombinant BCMAmut proteins was evaluated via surface plasmon resonance (SPR). HEK-293 cell lines were engineered to express wild type (WT) BCMA or individual BCMAmuts. Binding was assessed by flow cytometry. Co-culture assays were run with BCMAmut HEK cells, healthy donor CD3+ T cells, and BCMAxCD3 TCEs to evaluate killing potency (CellTiterGlo) and T cell activation (flow cytometry). The human tissue samples were sourced from vendors who follow protocols that are reviewed and approved by appropriate regulatory and ethics authorities.

Results

AlphaFold models predicted S30del and P33S would not influence elra binding, while R27P and P34del might reduce binding due to structural changes (**Figures 3-6, section A**). SPR-derived measurements confirmed the AlphaFold predictions and showed decreased binding affinity for both elra and tec to R27P and P34del (**Table 1**).

Both elra and tec cell binding and cytotoxicity were decreased by R27P and P34del (**Figures 3 & 6, sections C & D**). Neither elra nor tec binding or cytotoxicity was decreased by P33S (**Figure 5, sections C & D**). S30del minimally impacted elra binding affinity (K_D 0.168 nM, 4-fold weaker than to WT BCMA), while tec binding affinity was substantially reduced (K_D 145 nM, 711-fold weaker than to WT BCMA) (**Figure 4, sections B & C**). Correspondingly, elra T cell-mediated killing with associated T cell activation was maintained toward S30del, while tec activity was severely diminished (**Figure 4, sections D & E**).

Figure 1. Characterization of BCMA antigen expression in engineered HEK-293 cell lines.

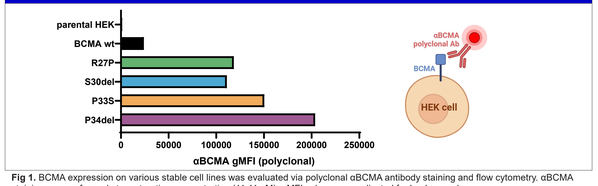


Fig 1. BCMA expression on various stable cell lines was evaluated via polyclonal αBCMA antibody staining and flow cytometry. αBCMA staining was performed at a saturating concentration (11.11 nM). gMFI values were adjusted for background.

Table 1. Kinetic constants for elranatamab and teclistamab with BCMA protein variants determined via SPR

	Affinity	Kinetics	
	K_D (nM) (SD)	k_a (x 10^6 $M^{-1}s^{-1}$) (SD)	k_d (x 10^{-4} s^{-1}) (SD)
Elranatamab			
BCMA WT	0.046 (0.009)	4.15 (0.80)	1.89 (0.13)
BCMA R27P	2480 (300)	--	--
BCMA S30del	0.168 (0.023)	3.51 (0.47)	5.88 (0.17)
BCMA P33S	0.588 (0.066)	2.47 (0.28)	14.53 (0.25)
BCMA P34del	342 (35)	--	--
Teclistamab			
BCMA WT	0.204 (0.047)	3.30 (0.75)	6.73 (0.25)
BCMA R27P	> 5000 (N/A)	--	--
BCMA S30del	145 (6)	--	--
BCMA P33S	1.89 (0.21)	2.51 (0.16)	47.5 (4.2)
BCMA P34del	148 (14)	--	--

k_a = Association rate constant; k_d = Dissociation rate constant; K_D = Equilibrium dissociation constant. Reported K_D values are the ratio of the means of the kinetic rate constants, K_D=K_d/K_a or means of the K_Ds obtained by steady-state fitting for experimental triplicates. K_D std. deviation values were calculated using the propagation of error principle as described in Taylor, 1997.

Figure 2. BCMA WT. Both elranatamab and teclistamab demonstrate robust binding and cytotoxicity

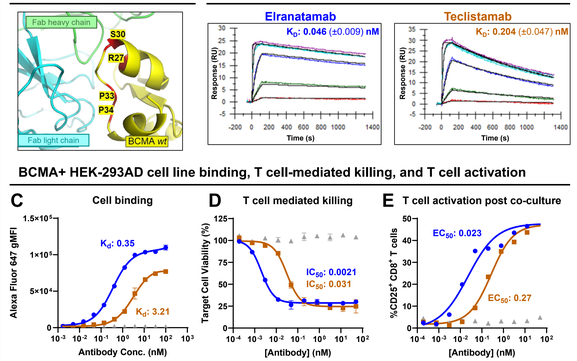


Figure 3. BCMA R27P reduced binding and cytotoxicity of both elranatamab and teclistamab

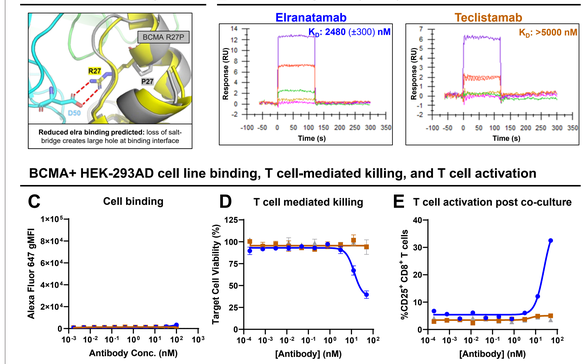


Figure 4. BCMA S30del reduced binding and cytotoxicity of teclistamab but not elranatamab

Figure 5. BCMA P33S maintained binding and cytotoxicity for both elranatamab and teclistamab

Figure 6. BCMA P34del reduced binding and cytotoxicity of both elranatamab and teclistamab

Figs 2-6. A. AlphaFold modeling of the interaction between various BCMA protein variants and the αBCMA Fab parental to elra. WT BCMA is displayed in yellow; mutant BCMA protein is overlayed in grey. The αBCMA Fab heavy chain is shown in green, and the light chain in blue. **B** Representative SPR sensorgrams of elra or tecli binding to individual BCMA protein variants at 37°C. Plots are color-coded by analyte concentration **C** Antibody binding of elra or tecli to engineered BCMA+ HEK-293 cell lines; binding was detected with Alexa Fluor 647-labeled goat anti-human IgG. Representative plot from two separate experiments is shown. Values plotted as technical duplicates and are baseline-corrected. Apparent K_D values are noted for complete binding curves and were obtained via Binding – Saturation (One site – Total) nonlinear regression analysis on GraphPad Prism. **D** T cell-mediated killing in 64-hour co-culture assays with engineered HEK-293 cells and either elra or tecli. Pan-CD3+ T cells were isolated from 2 healthy donors. Representative plots from two or three separate experiments are shown. Data were normalized against viability of T cell + target cell alone and values are plotted as technical duplicates. IC_{50} values are noted for complete killing curves and were obtained via Dose-response – Inhibition ([Inhibitor] vs. response – Variable slope (four parameters)) nonlinear regression analysis on GraphPad Prism. **E** CD25+ CD8+ T cell frequencies post co-culture as a measure of T cell activation. Representative plots from two or three separate experiments are shown. Data plotted as single value from pooled technical duplicates. EC_{50} values are noted for complete curves and were obtained via Dose-response – Stimulation ([Agonist] vs. response – Variable slope (four parameters)) nonlinear regression analysis on GraphPad Prism.