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Characterization of linvoseltamab's BCMA binding epitope and efficacy against BCMA mutations in relapsed/refractory multiple myeloma

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INTRODUCTION

- Patient outcomes in relapsed/refractory multiple myeloma (RRMM) have improved significantly with the adoption of BCMA targeted CAR-T and CD3 bispecific antibodies (bsAb)
- One such therapy, linvoseltamab, is a fully human BCMA×CD3 bsAb which has demonstrated high and durable responses in RRMM
- Recent data from patients who relapsed following treatment with approved BCMA×CD3 bsAbs identified rare in-frame, single amino acid deletions or mutations in the extracellular domain of BCMA[1]
- Both teclistamab and elranatamab were shown to bind similar epitopes on BCMA, involving or proximal to the residues with identified mutations (R27P, S30del, P33S, P34del)[1,2]
- The R27P and P34del mutations in BCMA were reported to impair the binding and cytolytic activity of both teclistamab and elranatamab *in vitro*; the S30del mutation negatively impacted teclistamab activity[1]

AIMS

- Determine the structure of the linvoseltamab BCMA-binding Fab in complex with BCMA
- Identify the BCMA binding epitope of linvoseltamab in comparison with teclistamab and map the reported BCMA mutations identified in literature[1]
- Evaluate whether the *in vitro* activity of linvoseltamab is impacted by the presence of the identified mutations in BCMA in comparison with teclistamab

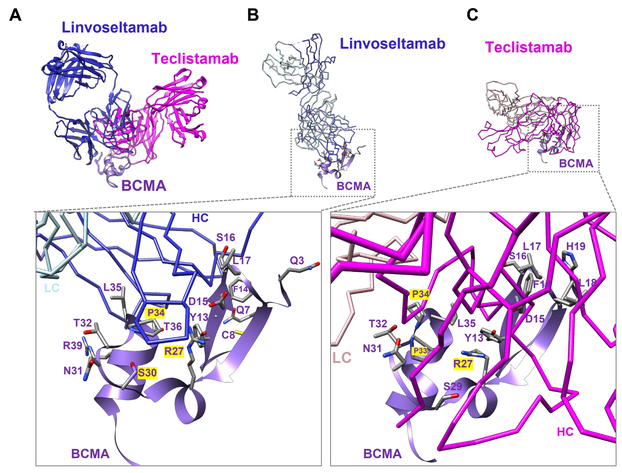
METHODS

- **Cryo-EM:** The anti-BCMA Fab fragments of linvoseltamab and teclistamab* were generated using the Fabricator enzyme. Anti-BCMA Fabs were complexed with the extracellular domain of BCMA (M1-A54) along with an anti-kappa light chain antibody Fab (linvoseltamab complex) or an anti-lambda light chain antibody Fab (teclistamab complex). Samples were frozen on UltrAufoil R1.2/1.3 grids using a Vitrobot Mark IV. Cryo-EM data were collected with a Titan Krios G3i microscope equipped with a K3 camera and processed using Cryosparc v2.14.2. Manual model building was carried out using Coot 0.8.9 and real space refinements were done in Phenix 1.17. Protein–protein interactions were analysed using MOE software
- **Cell-line generation:** HEK293 cells were engineered to express hCD20 and either wt BCMA or BCMA with patient-identified mutations via transfection or lentiviral transduction and antibiotic selection
- **Cell binding:** HEK293/hCD20 cells expressing wt or mutated BCMA cells were plated in 96-well plates and incubated with a titration of antibody, washed and then stained with an APC-conjugated secondary antibody and a viability dye and fixed. Fluorescence was measured by flow cytometry
- **Reporter bioassay:** Jurkat NFAT-Luc reporter cells were incubated in the presence of HEK293/hCD20 cells with wt or mutated BCMA and titration of antibody for 5 hours at 37°C. Reporter activity was detected as luminescence with OneGlo reagent (Promega)
- **Cytotoxicity assays:** Fluorescently labelled T cells were incubated with HEK293/hCD20 cells expressing wt or mutated BCMA cells at an effector-to-target cell ratio of 12.5:1 and serial dilutions of BCMA×CD3 bsAb, CD20×CD3 or non-TAA×CD3 control for 3 days at 37°C. Cytotoxicity and T-cell activation were assessed by flow cytometry

*Teclistamab used in these experiments was obtained from Pharmaceutical Buyers, Inc.

RESULTS

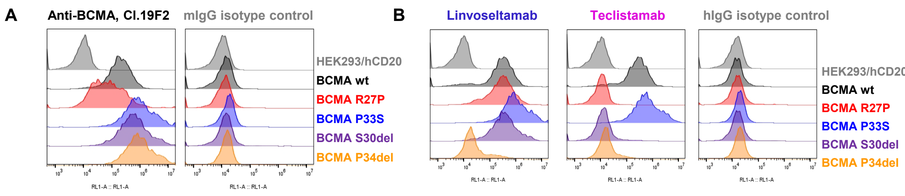
Figure 1. Linvoseltamab and teclistamab have distinct binding orientations on BCMA



D	Linvoseltamab	Teclistamab
	Q3	
	Q7	
	C8	
	Y13	Y13
	F14	F14
	D15	D15
	S16	S16
	L17	L17
		L18
	R27	R27
		S29
	S30	
	N31	N31
	T32	T32
		P33
	P34	P34
	L35	L35
	T36	
	R39	

(A) Overlay of Fab complexes for BCMA/linvoseltamab and BCMA/teclistamab structures. **(B)** 3.4 Å cryo-EM structure of BCMA/linvoseltamab Fab complex with BCMA and the Fab shown in cartoon form and C-alpha ribbon traces, respectively. BCMA residues involved in linvoseltamab binding are shown in stick representation. The mutated BCMA residues R27 and S30 are contacting linvoseltamab but they are both located at the edge of the binding interface. P34 is involved in linvoseltamab binding and sits in the centre region of the interface. P33 does not contribute to linvoseltamab binding. **(C)** Cryo-EM structure of BCMA/teclistamab Fab complex with BCMA and the Fab shown in cartoon form and C-alpha ribbon traces, respectively. BCMA residues involved in teclistamab binding are shown in stick representation. C is shown in the same orientation as B. The mutated BCMA residues R27 and P34 are contacting teclistamab and they are both located in the centre region of the binding interface. P33 contributes minimally to teclistamab binding. S30 does not directly contact teclistamab. **(D)** BCMA binding epitopes of linvoseltamab and teclistamab revealed by cryo-EM structures. BCMA residues with observed mutations are highlighted in yellow in the table and in the zoomed-in regions of panels 1B and 1C.

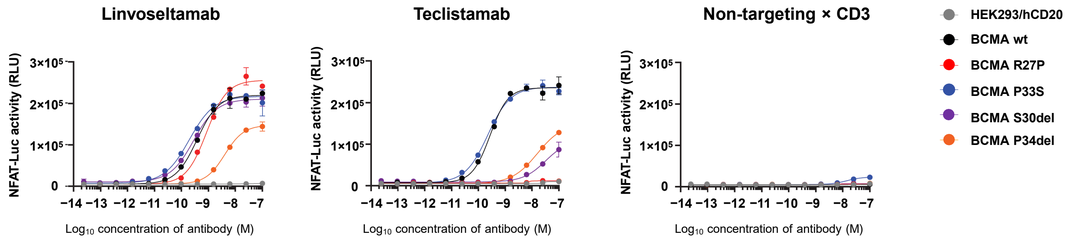
Figure 2. Linvoseltamab retains binding to BCMA R27P, S30del and P33S



C	Binding EC_{50} (nM)	
Cell line	Linvo	Tecli
BCMA wt	2.71	1.62
BCMA R27P	79.9	ND
BCMA P33S	8.47	11.8
BCMA S30del	16.6	ND
BCMA 34del	ND	ND

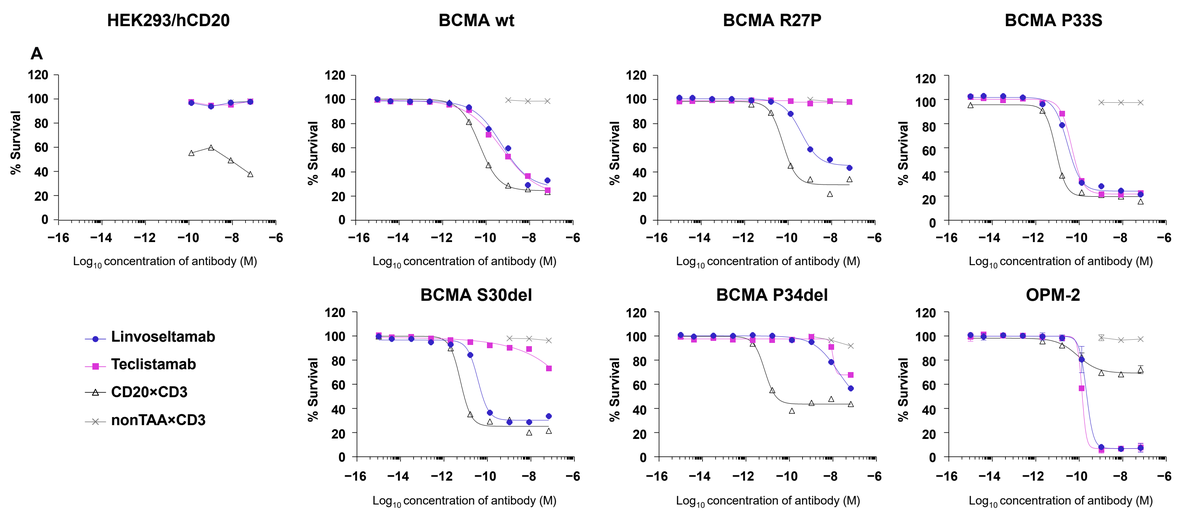
(A) Histogram plots from cell staining with 100nM anti-BCMA clone 19F2 or mouse IgG isotype control. BCMA is expressed on all engineered cells. **(B)** Histogram plots from staining with 100 nM of linvoseltamab, teclistamab or human IgG isotype control. Linvoseltamab binds to BCMA R27P, P33S and S30del comparably to wt. Teclistamab has significantly reduced binding to R27P, S30del and P34del. **(C)** Table of EC_{50} values (nM) derived from non-linear regression based on the cell staining experiment.

Figure 3. Linvoseltamab activates Jurkat T-cell reporter activity on BCMA with mutations identified from patients

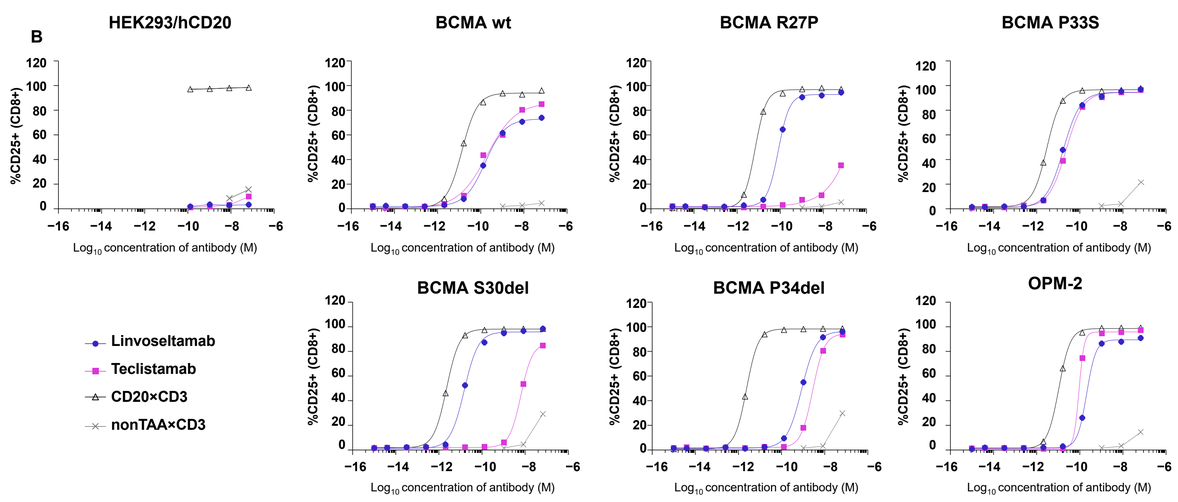


Linvoseltamab activates Jurkat cells in the presence of HEK293-expressing wt BCMA or each of the BCMA mutations tested, with reduced activity on BCMA P34del. Teclistamab has reduced activity on BCMA S30del and P34del, with no activity against R27P.

Figure 4. Linvoseltamab maintains cytotoxic activity against cells with BCMA mutations R27P, S30del and P33S



(A) Linvoseltamab, but not teclistamab, maintained cytotoxic activity against cells expressing BCMA with the R27P and S30del mutations. Both antibodies maintained their activity against cells with BCMA mutation P33S and showed reduced activity in cells with BCMA mutation P34del.



(B) Linvoseltamab, but not teclistamab, activates T cells in the presence of cells expressing BCMA with the R27P and S30del mutations. T-cell activation mediated by linvoseltamab and teclistamab correlated with their cytotoxic activity. Linvoseltamab-mediated T-cell activation was not impacted by mutations R27P, P33S and S30del and was reduced in cells with BCMA mutation P34del.

CONCLUSIONS

- Linvoseltamab contacts a total of 16 BCMA residues which largely overlap with teclistamab and the reported elranatamab epitope[1,2]
- However, linvoseltamab also binds to the N-terminal end of BCMA, suggesting that it is tilted toward the BCMA N-terminus with a distinct binding orientation
- While both teclistamab and elranatamab are susceptible to R27P-mediated resistance[1], linvoseltamab maintains binding and cytolytic activity against the R27P mutation
- Linvoseltamab also maintains binding and cytolytic activity against the S30del mutation which impairs the activity of teclistamab
- This suggests that linvoseltamab may be less susceptible to resistance mechanisms that involve the R27P and S30del mutations, potentially improving patient outcomes for patients harbouring these clones

ABBREVIATIONS

APC, alkaline phosphatase-conjugated; BCMA, B-cell maturation antigen; bsAb, bispecific antibody; CAR-T, chimeric antigen receptor T cell; CD, cluster of differentiation; cryo-EM, cryogenic electron microscopy; EC_{50}, half maximal effective concentration; Fab, fragment antigen-binding; HC, heavy chain; hCD20, human cluster of differentiation 20; HIgG, human immunoglobin G; IgG, immunoglobulin G; LC, light chain; mIgG, mouse immunoglobin G; MOE, molecular operating environment; ND, not detected; NFAT-luc; nuclear factor of activated T-cells–luciferase; OPM-2, human multiple myeloma cell line; TAA, tumour-associated antigen; wt, wild-type.

REFERENCES

1. Lee H, et al. *Nat Med.* 2023;29(9):2295–2306
2. Josic M, et al. *ASCO Congress.* 2024, Poster #7546.

ACKNOWLEDGEMENTS

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