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Characterization of the BCMA epitope bound by BCMA-CD3 T cell engager elranatamab.

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Objectives

Herein we characterize the BCMA binding affinity, evaluate the impact on BCMA binding to BAFF and APRIL interactions, and identify the BCMA binding epitope of elranatamab. We also generate a model from crystal structure data to map reported BCMA mutations identified in patients.

Conclusions

- Elranatamab binds with high affinity to an epitope on human BCMA that competes with lower affinity human APRIL and BAFF ligand binding.
- Elevated levels of APRIL or BAFF ligands, similar to those reported in MM patients, do not impact elranatamab mediated-tumor cell killing.
- Alpha-fold modeling indicates that identified BCMA mutations in RRMM patients lie along the BCMA/antibody binding interface.
- While linear epitopes suggest large overlap for elranatamab vs. teclistamab (**Table 1**), the mutations differentially impact drug potency. Teclistamab potency was decreased by three of four mutations/deletions (R27P, S30del, P34del), while elranatamab potency was only decreased by two (R27P, P34del).[8]
- While S30 is identified in the elranatamab, but not teclistamab epitope, S30del decreases teclistamab, but not elranatamab potency.[8] Thus, impacts of novel mutations on the 3D, rather than linear epitope, should be explored by modeling and functional assays. These data suggest elranatamab may prove better treatment for patients with S30del alterations.

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Abbreviations: **APRIL**: a proliferation-inducing ligand; **BAFF**: B cell activating factor; **BCMA**: B cell maturation antigen; **CAR-T**: chimeric antigen receptor T cell; **CDR**: complementarity determining region; **E:T**: effector to target ratio; **EMA**: European Medicines Agency; **FDA**: Food & Drug Administration; **IMID**: immunomodulatory drug; **LUC**: luciferase; **MM**: multiple myeloma; **PI**: proteasome inhibitor; **RRMM**: relapsed/refractory multiple myeloma; **SPR**: surface plasmon resonance

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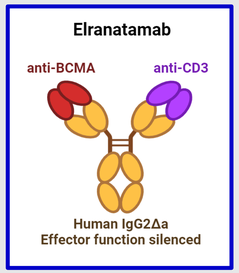
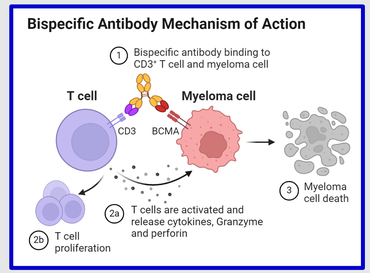
Background

- BCMA is a cell-surface receptor on plasma cells that is overexpressed in multiple myeloma.[1]
- BCMA-targeting modalities, including T cell-engaging bispecifics and CAR-Ts, have shown significant clinical benefit in myeloma.[1]
- Elranatamab is a BCMA-CD3 bispecific antibody that binds BCMA on myeloma tumor cells and CD3 on T cells. Dual binding to BCMA and CD3 causes T cell activation, cytokine release, and tumor cell killing.[2,3]
- Elranatamab has received an accelerated FDA[4] and conditional EMA[5] approval in 2023 for relapsed/refractory multiple myeloma (RRMM, post at least 1 treatment with IMiD, PI, and anti-CD38) and is currently in clinical development for earlier lines of treatment.





Methods

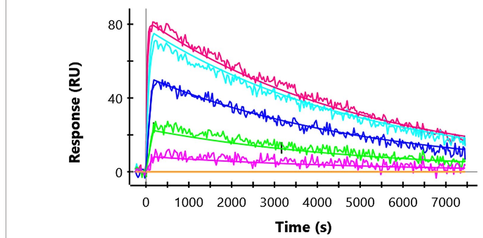
- Elranatamab binding affinity to recombinant human BCMA and characterization of BAFF and APRIL interactions with elranatamab-bound BCMA were evaluated via SPR at 37˚C with a classical sandwich format.
- In vitro tumor killing assays were performed with CD3+ T cells from 3 healthy donors and the relatively low BCMA-expressing Molp8/LUC cell line, at an E:T of 5:1 and 64-hour incubation period.
- The elranatamab BCMA binding epitope was identified by a co-crystal structure with a parental anti-BCMA Fab that has identical CDR sequences to elranatamab.
- Alpha-fold modeling was used to map recently identified rare BCMA mutations on the antibody/BCMA interface.

Results

ELRANATAMAB BINDING TO HUMAN BCMA COMPETES WITH BAFF AND APRIL LIGAND BINDING

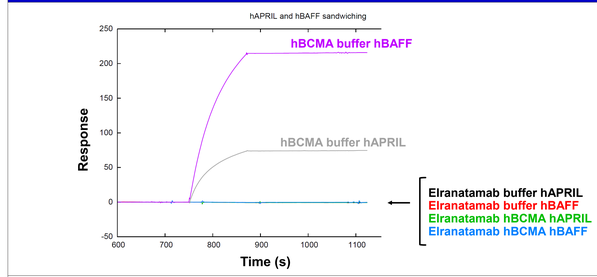
- Human APRIL and BAFF are ligands for the BCMA receptor.[6] They circulate as trimers and upon binding mediate increased cell proliferation and survival via NFkB signaling.[6]
- Elranatamab binds BCMA with high affinity (**Figure 1**). Binding competition assays determined elranatamab binding to BCMA blocks human APRIL or BAFF BCMA binding (**Figure 2**).
  - APRIL has an affinity of 16 nM for human BCMA.[6] BAFF has an affinity of 1.6 µM for human BCMA.[6]

Figure 1. Elranatamab binds to BCMA with high affinity (K_D ~38 pM).



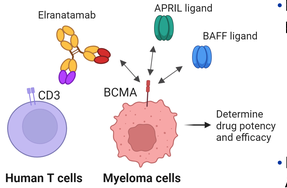
Representative sensorgram of SPR data evaluating binding of elranatamab to human BCMA at 37ºC. Elranatamab concentrations of 0.123, 0.370, 1.11, 3.33, and 10.0 nM were evaluated. Kinetic fits are shown.

Figure 2. Elranatamab blocks APRIL and BAFF binding to BCMA.



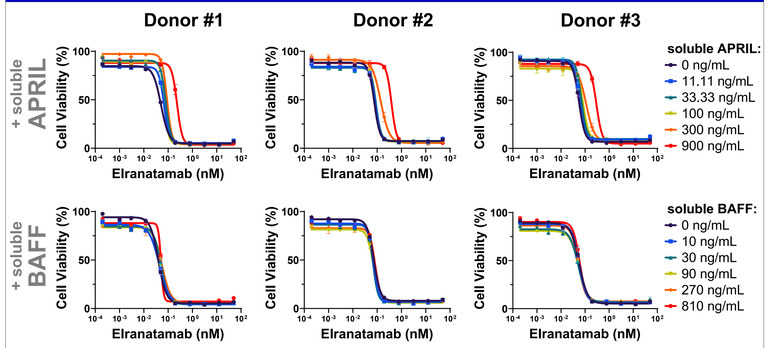
Blank-subtracted sensorgram of hBAFF and hAPRIL sandwiching interaction. Data are labeled by "Capture" "First Analyte" "Second Analyte." Sensorgram is cropped to only show "Second Analyte" assay step.

ELEVATED LEVELS OF APRIL OR BAFF DO NOT IMPACT ELRANATAMAB TUMOR KILLING POTENCY



- Human APRIL and BAFF circulate at elevated levels in MM patients.[7]
  - Soluble APRIL circulates at a mean concentration of ~70 ng/mL (0 - ~250 ng/mL).[7] Titrations from 11.11 - 900 ng/mL were tested.
  - Soluble BAFF circulates at a mean concentration of ~15 ng/mL (0 - ~600 ng/mL).[7] Titrations from 10 - 810 ng/mL were tested.
- In vitro tumor killing co-culture demonstrated no impact of soluble APRIL or BAFF at physiological levels on elranatamab potency (**Figure 3**).

Figure 3. *In vitro* tumor killing assays demonstrate elevated levels of human BAFF or APRIL do not impact elranatamab-mediated killing of MM tumor cells.



A CO-CRYSTAL STRUCTURE OF BCMA AND THE PARENTAL FAB OF ELRANATAMAB IDENTIFIES THE BCMA EPITOPE

- A co-crystal structure of an anti-BCMA Fab parental to elranatamab (same CDR sequences, **Figure 4**) was solved in complex with human BCMA, and the BCMA epitope binding elranatamab was identified (**Table 1**).
- The co-crystal structure was referenced to analyze four BCMA protein deletions or mutations recently identified in RRMM patients and suggested to potentially impact T cell engager function.[8]
- Alpha-fold modeling tool was used to model BCMA mutant locations in the structure (**Figure 5**).

Figure 4. Alignment of complementary determining regions (CDRs) of parental anti-BCMA Fab and elranatamab shows 100% sequence identity.

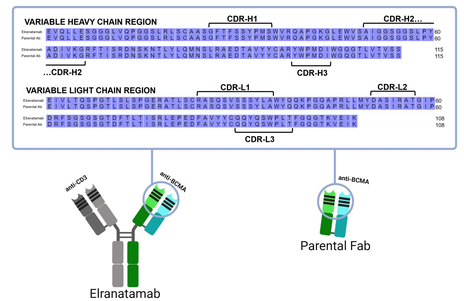
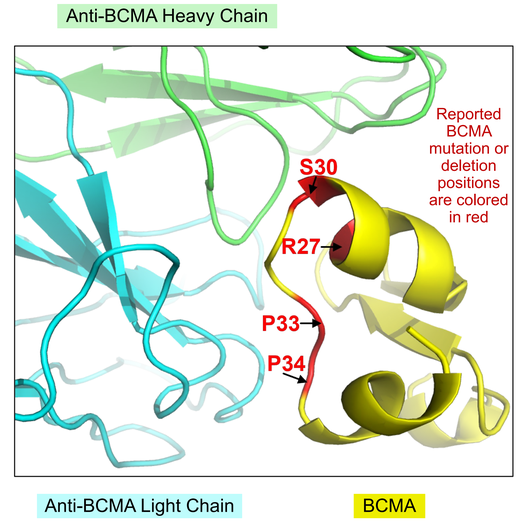


Table 1. BCMA binding epitope of anti-BCMA bispecific antibodies compared to key ligands.

Elranatamab	Teclistamab[9]	APRIL[10,11]	BAFF[10,12]
Y13	Y13	Y13	Y13
F14	F14	F14	F14
D15	D15	D15	D15
S16	S16		
L17	L17	L17	L17
	L18	L18	L18
	H19		H19
		I22	I22
L26	L26	L26	L26
R27	**R27**	**R27**	**R27**
S29			
S30		**S30**	
N31	N31		
T32	T32	T32	
P33	**P33**		
P34	**P34**	**P34**	**P34**
	L35		

BCMA residues identified in epitopes with various binding partners. BCMA mutation or deletion positions colored red.

Figure 5. Mapping the reported BCMA mutations on the co-structure of BCMA in complex with the parental Fab of elranatamab.



Alpha-fold modeling of reported BCMA mutations in complex with anti-human BCMA Fab.