Overcoming Mechanisms of Escape from Treatments for Multiple Myeloma by ISB 2001, a first-in-Class Trispecific BCMA and CD38 targeted T Cell Engager

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SUMMARY

Despite remarkable clinical advances following the approval of many novel drugs, or their combinations, multiple myeloma (MM) remains uncurable. Thus, relapse is currently inevitable and many patients progress and become triple and penta-refractory[1]. It is therefore crucial to develop innovative therapeutic options for patients with relapsed/refractory MM. Even though treatments targeting CD38[2] or BCMA[3, 4, 5] have extended overall survival, durable responses are still limited potentially due to target downregulation observed for both CD38 and BCMA[6, 7]. Simultaneous targeting of two antigens has been previously proposed as a promising approach to improve binding to tumor cells, enhance tumor cell killing and therefore prevent tumor escape associated with antigen downregulation.

Here, we propose to simultaneously target BCMA and CD38 using ISB 2001, a first-in-class trispecific molecule based on the BEAT™ (Bispecific Engagement by Antibodies based on the TCR) technology.

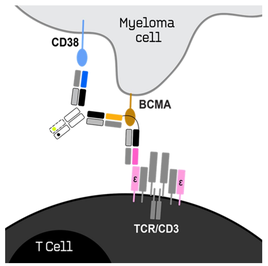
In summary, we have demonstrated that:

- Binding of two tumor-associated antigens increases the binding avidity and the potency of ISB 2001 compared to other BCMA-targeted T cell engagers. Importantly, ISB 2001 is less affected by the presence of soluble factors, such as sBCMA and APRIL, compared to other benchmarks.
- ISB 2001 shows superior killing potency relative to the combination of daratumumab and teclistamab.
- The half-life of ISB 2001 in a huFcRn Tg mouse model is 7.6 days.
- *In vivo*, ISB 2001 induces complete remission of all treated mice (8/8) compared to teclistamab, which only achieved partial tumor control.
- ISB 2001 shows superior cytotoxicity *ex vivo* compared to teclistamab at four different MM disease stages based on the assays with the primary patient tumor samples.

The enhanced therapeutic potential of ISB 2001 presented here supports the clinical development of ISB 2001. First in human administration is planned for first half of 2023.

KEY ATTRIBUTES

- Three proprietary antigen-binding arms: CD3ε on T cells; BCMA and CD38 on MM cells
- Heterodimerisation based on the BEAT platform in a TREAT® format
- Binding arms derived from synthetic phage display library with common light chain (Vκ3-15 + IgkJ1)
- Increased binding specificity to MM cells due to enhanced avidity-based binding of anti-BCMA and anti-CD38 Fab domains



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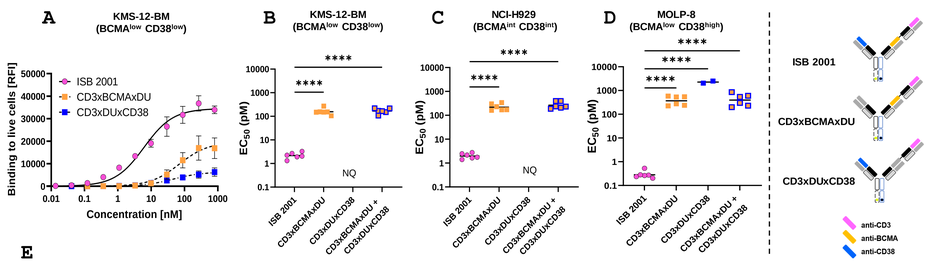
ISB 2001 is Designed to Mediate Potent MM Cell Killing via Dual Targeting Avidity-Driven Tumor Binding



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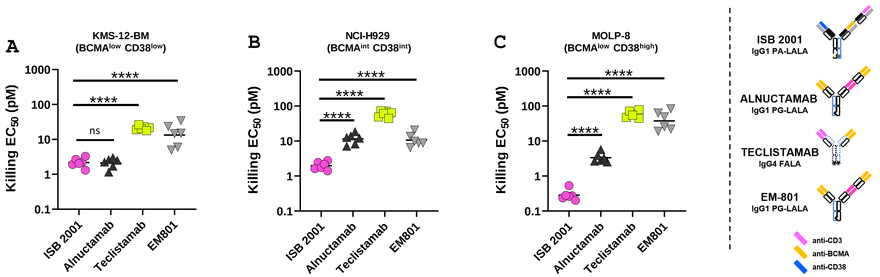
Expression sABC	CD38 expression (sABC)	BCMA expression (sABC)	Clinical case modelling
KMS-12-BM	LOW 28000	LOW 9000	Post treatment with daratumumab + teclistamab Newly Diagnosed or post IMIDs + PI
NCI-H929	MID 85000	MID 52000	
MOLP-8	HIGH 512000	VERY LOW 3200	Post BCMA targeted therapy

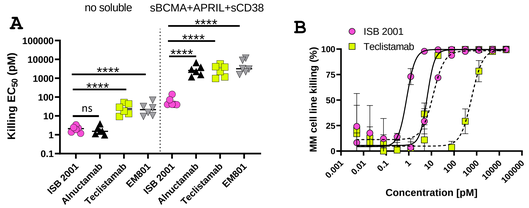
Cell Based Affinity measurements and Redirected Cell Lysis (RDL) of multiple myeloma cell lines presenting various levels of BCMA and CD38 tumor associated antigens to compare ISB 2001 killing potency to control molecules. (A) A dose-response of the antibodies starting at 800 nM was incubated with KMS-12-BM cell line and binding was detected using a secondary antibody anti-human Fc. Curve fitted using One-Site Specific Binding fitting. Symbols show the mean of 3 independent experiment ± SD. (B to D) Purified T cells were co-cultured with multiple myeloma cell lines to an E:T ratio of 5:1 for 48 hours in presence of a dose-response of each tested molecule. Graphs show the killing potency reflected by the 50% effective concentration (EC50) of each molecule for (B) KMS-12-BM, (C) NCI-H929 and (D) MOLP-8 cell lines. Each symbol represent an individual donor and horizontal bars show the average of 2-6 donors from 3 independent experiments. Stars show statistically significant differences in a Tukey post-hoc comparison performed after paired One-Way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). NQ: not quantifiable. (E) Expression levels of CD38 and BCMA tumor associated antigens in each cell line used and the clinical case modelling they represent.

ISB 2001 Possesses the Strong Cytotoxic Potency on Myeloma Cell lines Compared to Benchmarks



Redirected Cell Lysis (RDL) of multiple myeloma cell lines presenting various levels of BCMA and CD38 tumor associated antigens to compare ISB 2001 to CD3xBCMA benchmark molecules. Purified T cells were co-cultured with multiple myeloma cell lines to an E:T ratio of 5:1 for 48 hours in presence of a dose-response of each tested molecule. Graphs show the killing potency reflected by the 50% effective concentration (EC50) of ISB 2001 and CD3xBCMA engagers for (A) KMS-12-BM, (B) NCI-H929 and (C) MOLP-8 cell lines. Each symbol represent an individual donor and horizontal bars show the average of 6 donors from 3 independent experiments. Stars show statistically significant differences in a Tukey post-hoc comparison performed after paired One-Way ANOVA (****p<0.0001)

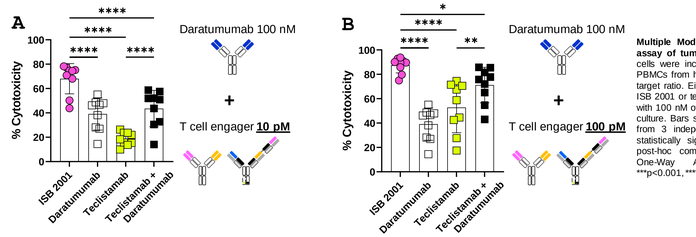
ISB 2001 Demonstrates Best Cytotoxicity in the Presence of Soluble Factors Compared to BCMA-Specific T Cell Engager Benchmarks



Tumor Killing EC50 (pM)	ISB 2001	Alnuctamab	Teclistamab	EM801
No Soluble Factors	2.1	1.9	27.4	27.1
sBCMA+APRIL+sCD38	62.5	3059.7	3037.6	5144.1
Fold Difference (sEC50/EC50)	30	1647	111	190

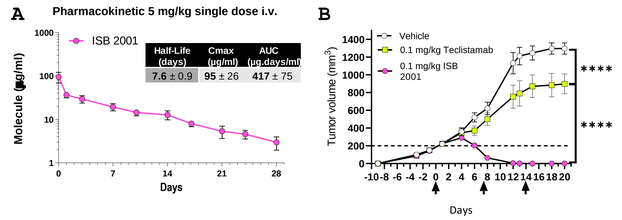
Redirected Cell Lysis (RDL) of multiple myeloma cell line in presence or absence of a combination of soluble factors found in blood of multiple myeloma patients. Isolated PBMCs from healthy donors were co-cultured with KMS-12-BM cell line to an E:T ratio of 5:1 for 48 hours in presence of a dose-response of each tested molecule. When mentioned, soluble factors were added (soluble BCMA (150 ng/ml), APRIL (100 ng/ml) and soluble CD38 (2.8 ng/ml)). (A) Killing potency reflected by the 50% effective concentration (EC50) of molecules without (left) and in presence of soluble factors (right). Horizontal bar show average of 5-6 donors from two individual experiments. Stars show statistically significant differences in a Tukey post-hoc comparison performed after paired One-Way ANOVA. (****p<0.0001) (B) Representative dose-response curves are displays for the treatment of ISB2001 and teclistamab in absence (solid line) and in presence of soluble factors. Curves fitted using Logistic 4PL nonlinear fitting with variable slope. Symbols show the mean of 5-6 donors ± SEM. (C) Average of tumor killing EC50 and fold difference in potency in presence of soluble factors.

ISB 2001 Demonstrates More Potent Cytotoxicity than the Combination of Teclistamab + Daratumumab



Multiple Modes of Action Killing (MMoAK) assay of tumor cell line KMS-12-BM. Tumor cells were incubated for 48 hours with human PBMCs from healthy donors at a 5:1 effector to target ratio. Either 10 pM (A) or 100 pM (B) of ISB 2001 or teclistamab alone or in combination with 100 nM of daratumumab were added to the culture. Bars show the mean ± SD of 9 donors from 3 independent experiments Stars show statistically significant differences in a Tukey post-hoc comparison performed after paired One-Way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

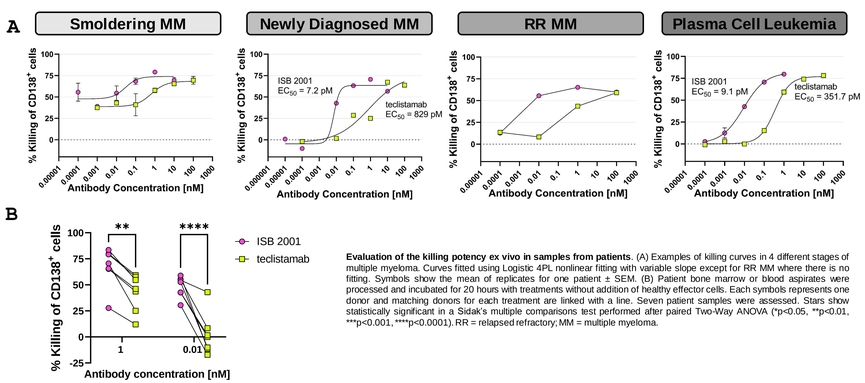
ISB 2001 shows a 7-day half-life *in vivo* and achieved 100% Complete Responses *in vivo* in a BCMA^low and CD38^low MM Murine Model



Half-Life (days)	Cmax (µg/ml)	AUC (µg.days/ml)
7.6 ± 0.9	95 ± 26	417 ± 75

In vivo pharmacokinetics and efficacy assessment. (A) ISB 2001 half-life was assessed in a huFcRn Tg mouse model (Tg32) (B) Efficacy was assessed in NSG-PBMC transfer mouse model by subcutaneous grafting of KMS-12-BM cell line and hPBMCs i.p. Arrows indicate when treatments were administered. Symbols show the mean ± SEM of 8 mice per group. Statistical evaluation was done by two-way ANOVA followed by Tukey's multiple comparison test (**p<0.01, ***p<0.001, ***p<0.0001)

ISB 2001 Exhibits Higher Potency *ex vivo* Compared to Teclistamab on MM Patient Samples



Evaluation of the killing potency ex vivo in samples from patients. (A) Examples of killing curves in 4 different stages of multiple myeloma. Curves fitted using Logistic 4PL nonlinear fitting with variable slope except for RR MM where there is no fitting. Symbols show the mean of replicates for one patient ± SEM. (B) Patient bone marrow or blood aspirates were processed and incubated for 20 hours with treatments without addition of healthy effector cells. Each symbols represents one donor and matching donors for each treatment are linked with a line. Seven patient samples were assessed. Stars show statistically significant in a Sidak's multiple comparisons test performed after paired Two-Way ANOVA (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). RR = relapsed refractory; MM = multiple myeloma.

ISB 2001 trispecific antibody demonstrated superior therapeutic properties compared to BCMA-targeted T cell engagers *in vitro*, *in vivo* and *ex vivo*. In addition, ISB 2001 demonstrates benefit over the clinically investigated combination of teclistamab + daratumumab.

First-in-human administration is expected to start in the first half of 2023