Synergistic targeting of multiple activating pathways with natural killer cell engagers

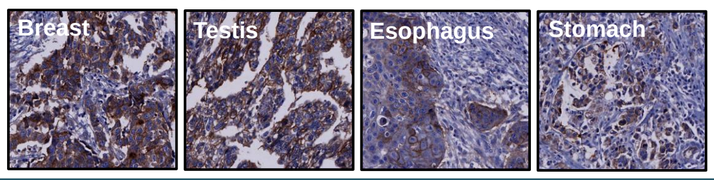
Tian Zhang, Matthew S. Faber, Erik Pong, Ke Liu, Nahee Kim, Matthew J. Bernett, Christine Bonzon, James Wieler, Juan E. Diaz, Kendra N. Avery, Elizabeth Henderson, William Yeh, Jing Qi, Su-Shin Hao, Rumana Rashid, Rena Bahjat, John R. Desjarlais, and Katrina Bykova

SITC 2023
Abstract #1193

Introduction

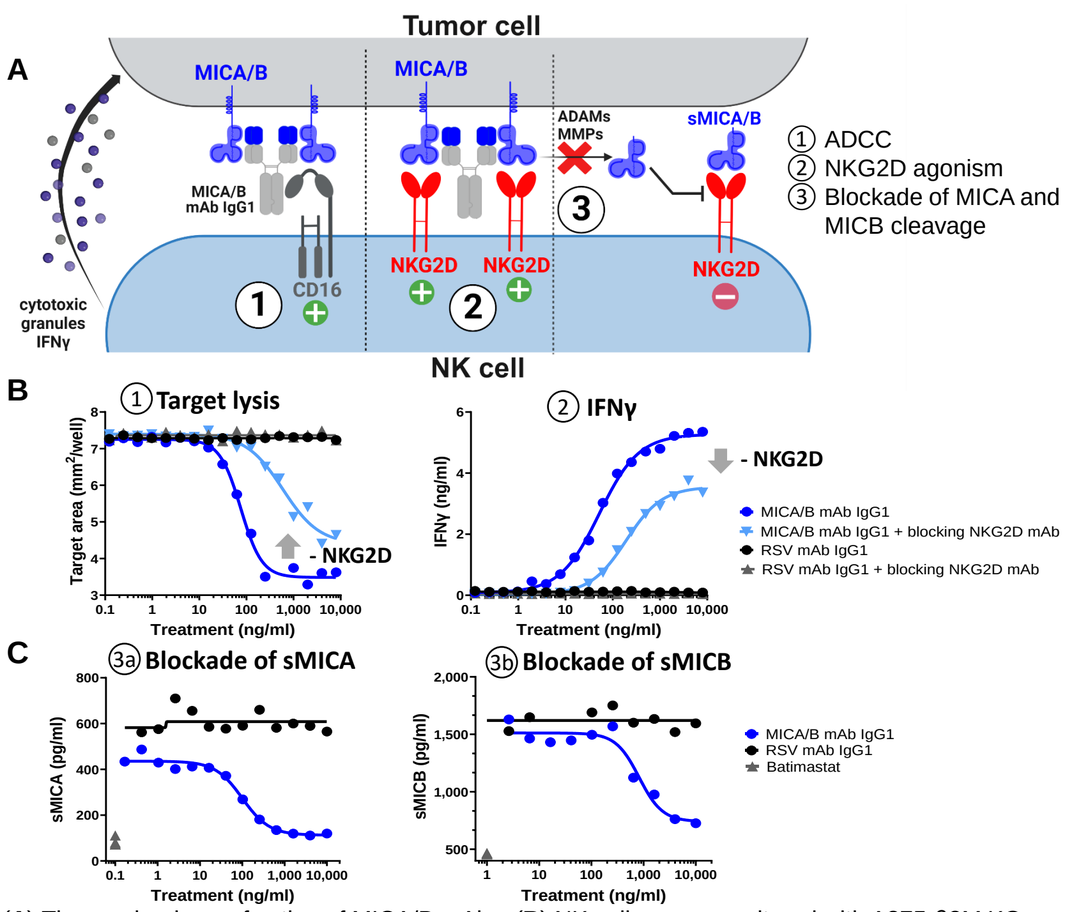
Tumor microenvironment induced antigens present a unique opportunity to effectively target diseased tissue over normal and to modulate the immune suppression they might elicit. MICA and MICB (MICA/B) are stress-induced antigens expressed in a range of cancers. MICA/B antigens are recognized by NKG2D, an activating receptor on NK and CD8+ T cells. While the membrane-bound form of MICA/B is immuno-stimulatory, the cleaved soluble form, found in the tumor microenvironment, prevents NKG2D mediated tumor cell recognition. To stop tumor escape and, concurrently, stimulate the innate and adaptive immune responses, we developed antibodies targeting MICA/B. Anti-MICA/B antibodies block proteolytic cleavage, increase MICA/B membrane surface densities, and activate NK and CD8+ T cells by bringing membrane bound MICA/B to NKG2D. To enhance the anti-tumor activities of MICA/B antibodies, we designed multi-specific NK cell engaging antibodies that simultaneously target MICA/B antigens and an orthogonal activating receptor NKp46.

MICA/B is expressed in multiple cancer indications



Multiple tumor tissue microarrays were stained with MICA/B antibodies. Pancreas, skin, lung, and ovary tumor tissues also showed tumor MICA/B expression.

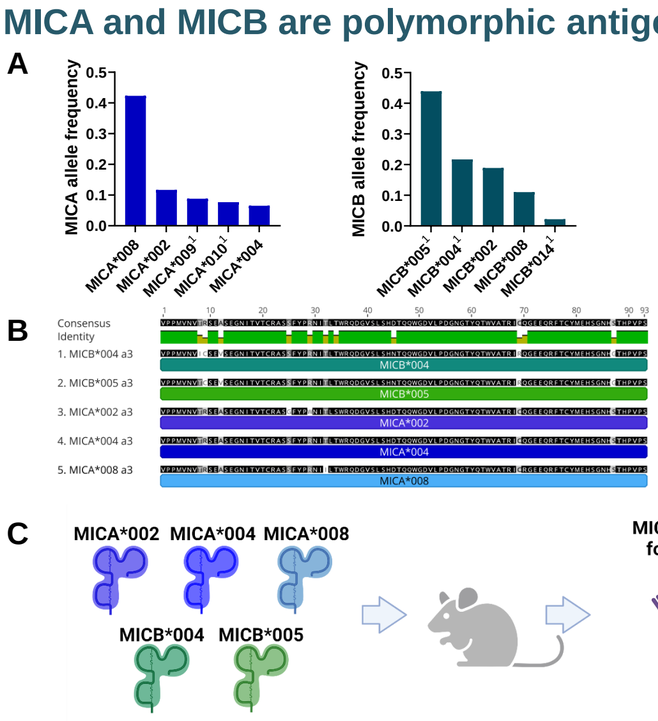
Mechanisms of action of MICA/B mAbs



(**A**) The mechanisms of action of MICA/B mAbs. (**B**) NK cells were cocultured with A375-β2M KO-RFP tumor cells and indicated treatments. Tumor cell growth was assessed with Incucyte. The RSV Fv was used as an isotype control. (**C**) CHO-MICA cells and CHO-MICB cells were incubated with indicated treatments. sMICA and B in the supernatant were measured by ELISA. Batimastat was used to inhibit cell surface proteases.

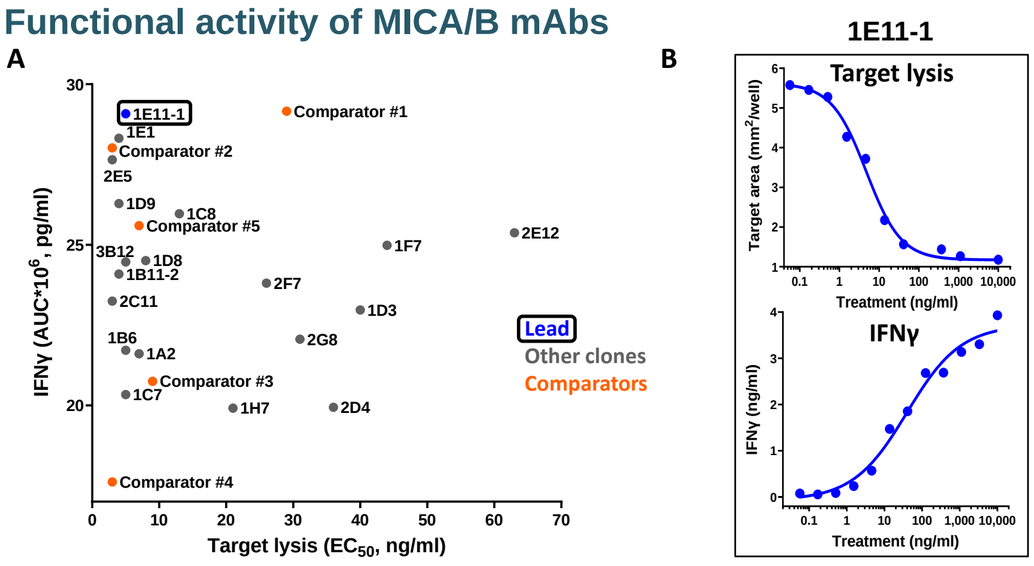
β2M KO, beta-2-microglobulin knockout; RSV, respiratory syncytial virus; sMICA/B, soluble MICA/B.

MICA and MICB are polymorphic antigens



(**A**) Population frequencies of MICA and MICB allelic variants. Alleles with higher frequencies are shown (Klussmeier A, et al. *Front Immunol.* 2020). [1]Allelic variants with ambiguous genotyping data. (**B**) α3 domain sequence alignment for higher frequency MICA and MICB variants. (**C**) Mouse immunization strategy for raising antibodies specific for multiple MICA and MICB alleles.

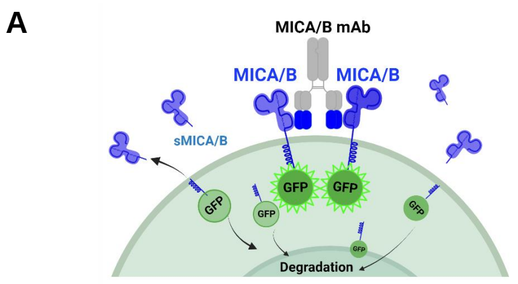
Functional activity of MICA/B mAbs



(**A**) Correlation between secreted IFNγ and target lysis of different MICA/B mAbs. NK cells were cocultured with MCF7-RFP tumor cells in the presence of MICA/B mAbs. Tumor cell growth was assessed with Incucyte. (**B**) Tumor target lysis and IFNγ production dose-response to the lead MICA/B clone 1E11-1.

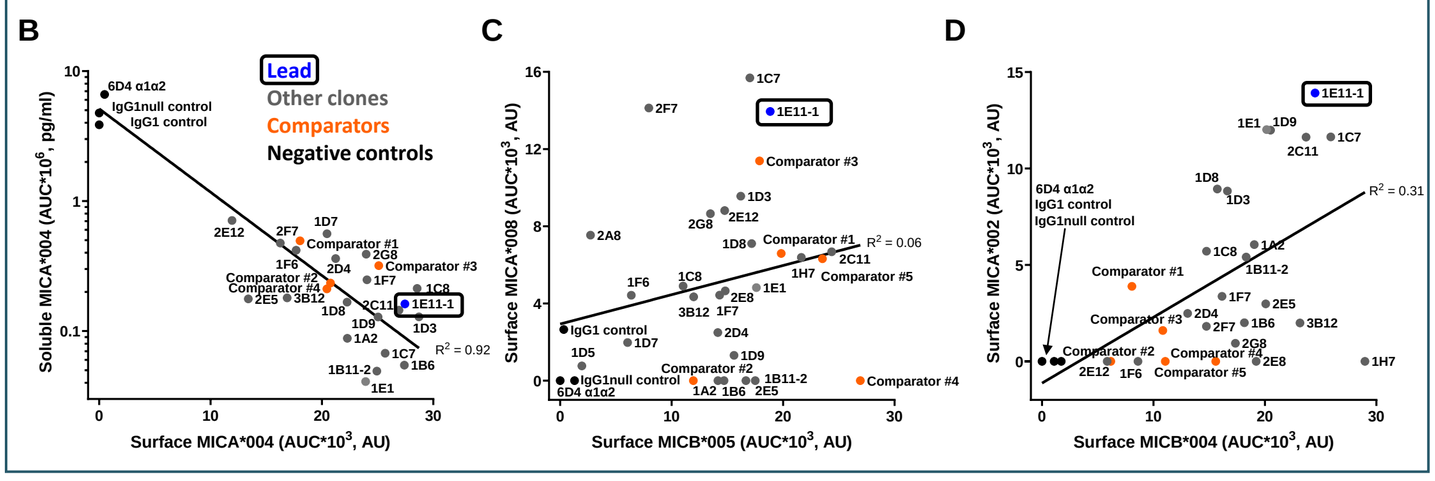
Contact: kbykova@xencor.com

MICA/B clones show activity on multiple MICA and MICB allelic variants

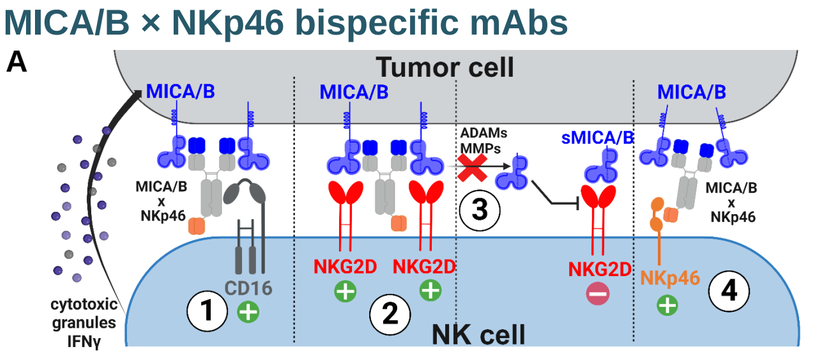


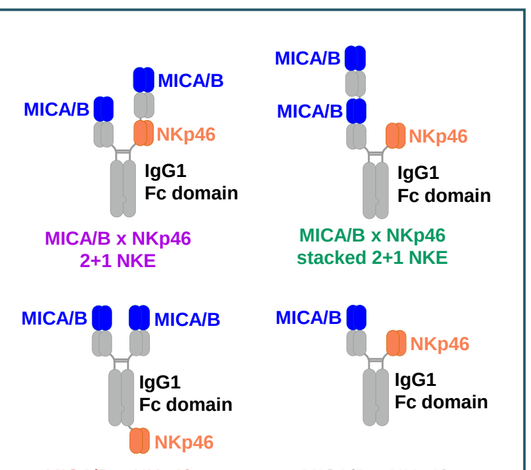
Surface MICA (MICB) density, measured via C-terminal GFP intensity, inversely correlates with membrane MICA (MICB) cleavage.

CHO cell lines expressing MICA and MICB allelic variants tagged with C-terminal GFP were incubated with MICA/B mAbs and controls. (**A**) Cartoon illustrating an inverse correlation between the surface MICA/B expression and soluble MICA/B. (**B**) Surface MICA*004-GFP upregulation inversely correlates with soluble MICA. The surface MICA/B-GFP was used to screen MICA/B mAb clones for their ability to prevent MICA and MICB shedding from multiple allelic variants. (**C**) Upregulation of surface MICB*005-GFP versus MICA*008-GFP on the surface of CHO cells. (**D**) Upregulation of surface MICB*004-GFP versus MICA*002-GFP on the surface of CHO cells.



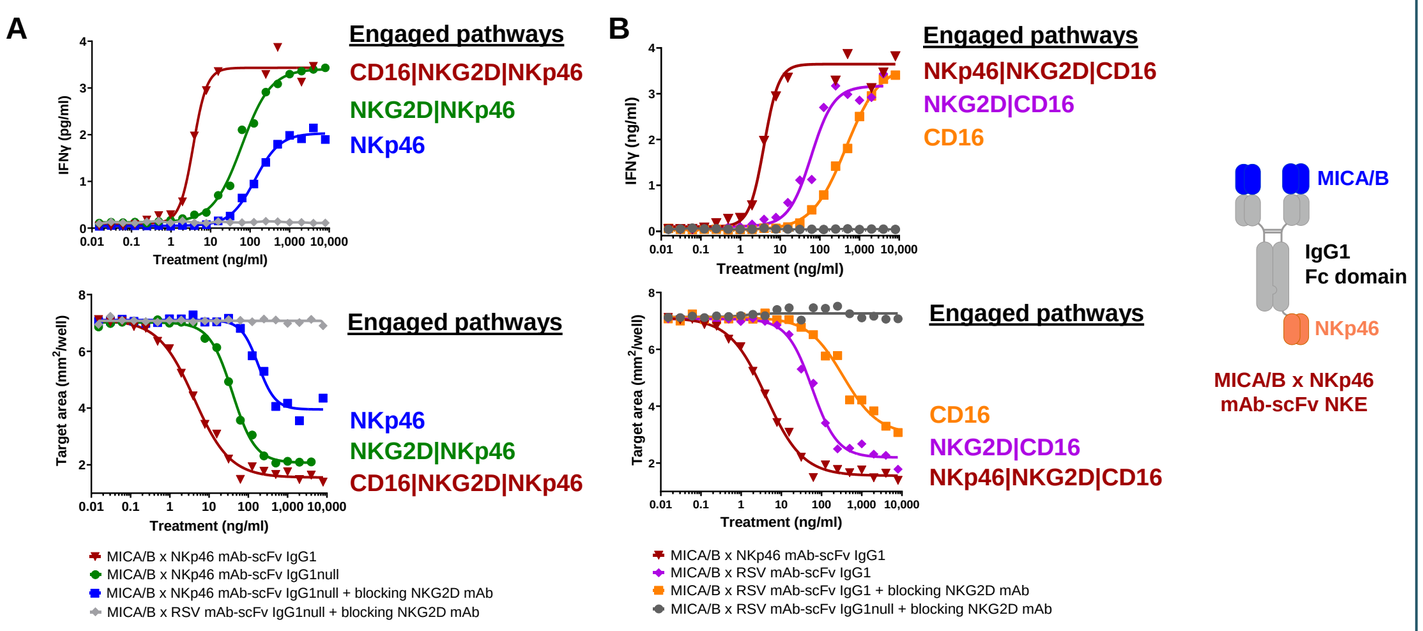
MICA/B × NKp46 bispecific mAbs





(**A**) The mechanisms of action of MICA/B × NKp46 mAbs.
(**B**) Structures of bispecific MICA/B × NKp46 mAbs.

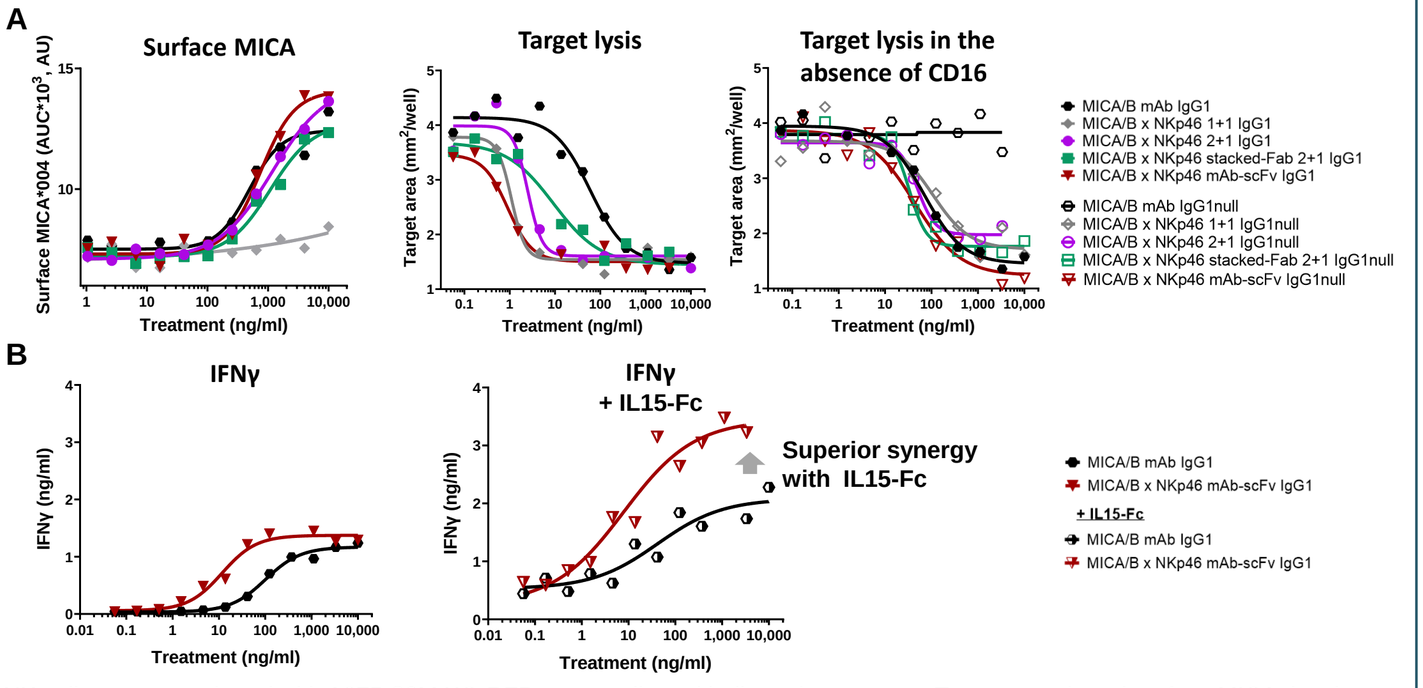
MICA/B × NKp46 mAb-scFv synergistically targets multiple activating pathways



NK cells were cocultured with A375-β2M KO-RFP tumor cells and indicated treatments with or without an NKG2D mAb that blocks MICA binding. Tumor cell growth was assessed with Incucyte. RSV was used as an isotype control.

β2M KO, beta-2-microglobulin knockout; RSV, respiratory syncytial virus.

MICA/B x NKp46 bispecific mAbs are superior to MICA/B mAbs



NK cells were co-cultured with A375-β2M KO-RFP tumor cells and indicated treatments. Tumor growth and surface MICA density were assessed with Incucyte. RSV was used as an isotype control.

β2M KO, beta-2-microglobulin knockout; RSV, respiratory syncytial virus.

Summary

- To address the polymorphic nature of MICA and MICB antigens, we developed antibodies that recognize multiple MICA/B allelic variants
- These MICA/B antibodies induce ADCC, agonize the NKG2D pathway, and block proteolytic cleavage of MICA and MICB
- We designed NK cell engagers that integrate multiple activating signals by adding NKp46 agonism to NKG2D pathway agonism and ADCC activity induced by MICA/B antibodies. Resulting NK engagers synergistically activate multiple pathways and show superior activity over MICA/B mAbs by retaining activity in the absence of CD16 engagement and agonizing NKp46 pathway