Abstract #2943

The Structural Basis for Inhibition of MICA Shedding and Anti-tumor Activity of the Monoclonal Anti-MICA/B Antibody, CLN-619

Kerry A. Whalen, Naveen K. Mehta, Catherine C. Henry, Kristan Meetze, Jennifer S. Michaelson, Patrick A. Baeuerle
Cullinan Oncology, Inc., Cambridge, MA, USA.

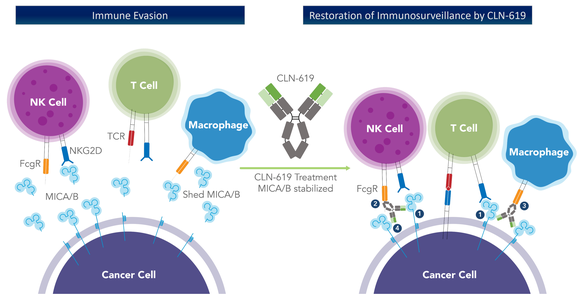
Background

- MICA/MICB are stress-inducible, surface glycoproteins that are up-regulated on a wide variety of human tumors and act as activating ligands for the Natural Killer Group 2 member D (NKG2D) receptor expressed on NK cells, NKT cells, CD8 and γ/δ T cells.[1, 2]
- While MICA/MICB expression marks cells for lysis by NKG2D-expressing immune cells, tumors can shed these proteins via cleavage by proteases present in the TME, thereby preventing immune cells from recognizing and destroying tumor cells.[3]
- High concentrations of shed MICA have been observed in sera from patients across multiple tumor types, where they correlate with poor survival.[4]
- MICA/MICB is highly polymorphic with >150 MICA and 47 MICB alleles in humans. Expression level, binding affinity to NKG2D, and degree of MICA/MICB shedding is thought to be allele-dependent.[5]

Features of CLN-619

- CLN-619 is a humanized IgG1 monoclonal antibody that specifically binds to both human MICA and MICB and to non-human primate orthologs.
- CLN-619 prevents the proteolytic release of MICA/MICB, thereby exposing tumor cells for immune cell destruction through both NKG2D-mediated and Fc-dependent effector mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent phagocytosis (ADCP).
- CLN-619 is currently being investigated in a Phase 1 clinical trial as a monotherapy and in combination with pembrolizumab for the treatment of patients with advanced solid tumors (NCT05117476).

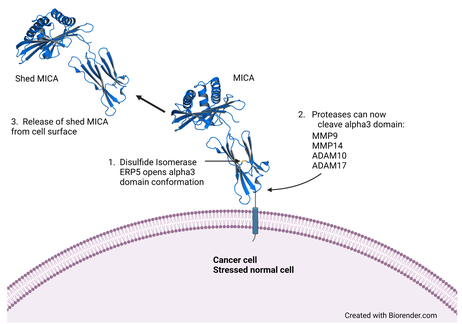
Figure 1: CLN-619 Multiple Modes of Action



1. Prevention of MICA/MICB shedding
2. Antibody-dependent cellular cytotoxicity (ADCC)
3. Antibody-dependent cellular phagocytosis (ADCP)
4. Enhanced binding of MICA to NKG2D

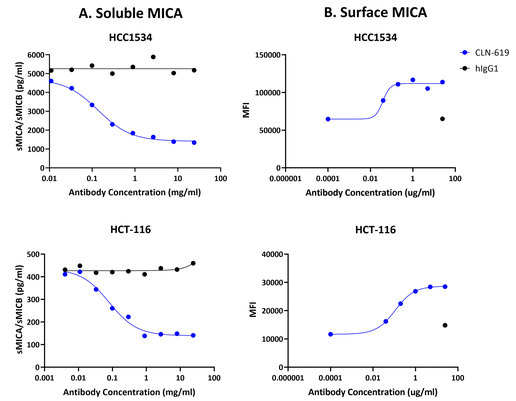
Results

Figure 2: Model of Proteolytic Shedding of MICA[6,7]



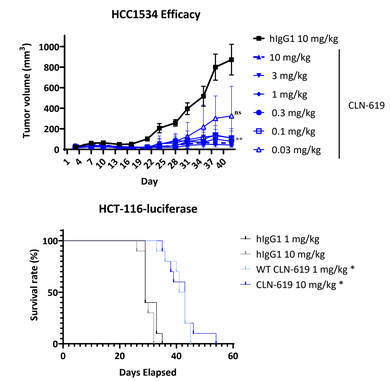
Upon isomerization of MICA by ERP5, proteases in the TME cleave cell surface MICA, enabling tumor cells to evade immune cell recognition. An antibody that binds to the alpha 3 domain of MICA may be able to prevent Erp5 activity and/or proteolytic shedding by MMPs, thus restoring immune-mediated tumor cell lysis. MICA structure: pbd 1je6.

Figure 3: CLN-619 Inhibits MICA/MICB Shedding In Vitro



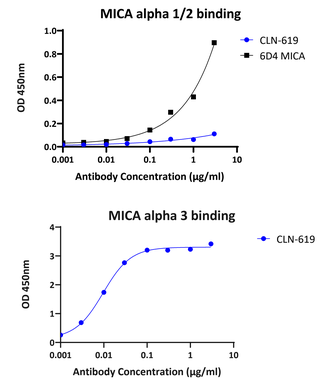
A-B) Cells were incubated with antibodies for 24 hours, N=2. A) MICA/MICB was measured in the cell supernatant by ELISA. B) MICA/MICB cell surface expression was measured by flow cytometry using the 6D4 antibody.

Figure 4: CLN-619 Is Efficacious In Vivo



All treatments were given IP 2x weekly starting on Day 1. A) BALB/c SCID mice were inoculated with HCC1534 lung cancer xenograft cells (N=10/group). Treatment was administered for six weeks. ns-not significant, ** P<0.005 by one-way ANOVA multiple comparisons. B) To model disseminated disease, CB17 SCID mice were inoculated IP with HCT-116-luciferase human CRC cells (N=10/group). Treatment was administered for eight weeks. All CLN-619 treated groups were significantly different from control by Log-Rank (*P<0.05).

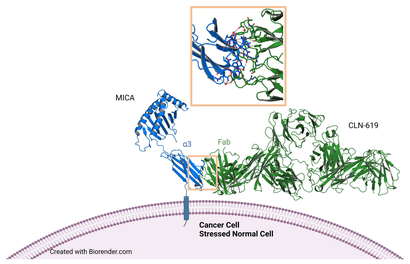
Figure 5: CLN-619 Binds alpha 3 Domain of MICA



ELISA plates were coated with MICA protein constructs comprising the alpha 1/2 domain or alpha 3 domain of human MICA1 and incubated with serially diluted CLN-619 or 6D4 control MICA antibody followed by detection with a labelled secondary antibody.

Figure 6: MICA/CLN-619 Co-crystal Structure

Exclusive binding of CLN-619 to the alpha 3 domain of MICA



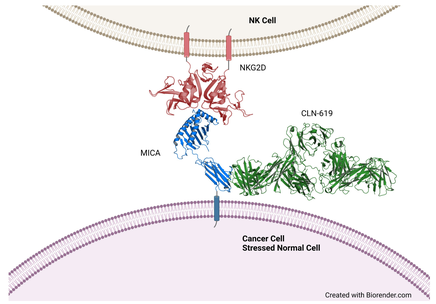
X-ray crystal structures of the human Fab antibody fragment CLN-619 in complex with the antigen MICA1 at 2.12 Å resolution. The binding interface of CLN-619 is a discontinuous epitope comprising 19 amino acid residues of the alpha 3 domain of MICA.

Figure 7: The CLN-619 Epitope is Well Conserved Across all MICA/B Alleles with ≥1% Frequency in the Population

Allele	Frequency(%)	Amino Acids in CLN-619 Epitope																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
*MICA1	0.7	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA2	11.7	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA4	6.5	Asn	Thr	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA7	4.7	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA8	42.3	Asn	Thr	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA9	8.8	Asn	Thr	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA10	7.7	Asn	Thr	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA11	1.7	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA12	2.3	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA16	1.9	Asn	Thr	Arg	Asp	Leu	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA17	3.4	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA18	3.7	Asn	Ser	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICA27	2.6	Asn	Thr	Arg	Asp	Val	Ser	Leu	Ser	His	Asp	Thr	Gln	Arg	Ile	Cys	Glu	Gln	Arg	Thr
MICB2	*18.9*	*Asn*	*Thr*	*Arg*	*Asp*	*Val*	*Ser*	*Leu*	*Ser*	*His*	*Asn*	*Thr*	*Gln*	*Arg*	*Ile*	*Arg*	*Glu*	*Gln*	*Arg*	*Thr*
MICB4	*21.7*	*Asn*	*Thr*	*Arg*	*Asp*	*Val*	*Ser*	*Leu*	*Ser*	*His*	*Asn*	*Thr*	*Gln*	*Arg*	*Ile*	*Arg*	*Glu*	*Gln*	*Arg*	*Thr*
MICB5	*43.9*	*Asn*	*Thr*	*Arg*	*Asp*	*Val*	*Ser*	*Leu*	*Ser*	*His*	*Asn*	*Thr*	*Gln*	*Arg*	*Ile*	*Arg*	*Glu*	*Gln*	*Arg*	*Thr*
MICB8	*11*	*Asn*	*Thr*	*Arg*	*Asp*	*Val*	*Ser*	*Leu*	*Ser*	*His*	*Asn*	*Thr*	*Gln*	*Arg*	*Ile*	*Arg*	*Glu*	*Gln*	*Arg*	*Thr*
MICB14	*2.2*	*Asn*	*Thr*	*Arg*	*Asp*	*Val*	*Ser*	*Leu*	*Ser*	*His*	*Asn*	*Thr*	*Gln*	*Arg*	*Ile*	*Arg*	*Glu*	*Gln*	*Arg*	*Thr*

Sequence analysis of the CLN-619 discontinuous epitope for MICA/B alleles with >1% frequency in the population reveals high homology with conservative amino acid changes shown in orange. Binding of CLN-619 to all MICA allelic variants listed in the table and to the MICB canonical allelic variant was confirmed by Octet, ELISA and/or Luminex.[8]
* Denotes canonical alleles.

Figure 8: Interaction of CLN-619/MICA/NKG2D



CLN-619 binds to a highly conserved region of the alpha 3 domain of MICA/B and prevents shedding. CLN-619 binding to MICA does not interfere with the receptor-ligand interaction.

Conclusions

- CLN-619 prevents proteolytic release of MICA/MICB from cells resulting in increased cell surface expression of MICA/MICB.
- CLN-619 exhibits potent *in vivo* anti-tumor activity in mice bearing MICA/MICB-expressing human tumor xenografts.
- CLN-619 binds to a discontinuous epitope comprising 19 amino acids in the alpha 3 domain of MICA.
- The CLN-619 epitope is highly conserved, resulting in broad MICA/B allelic coverage.
- A Phase 1 clinical trial with CLN-619 as monotherapy and in combination with pembrolizumab is in progress.

References

1. Zingoni A, et al. Front. Immunol, 2018
2. Lanier LL, et al. Cancer Immunol Res, 2015
3. Chitadze G, et al. Scan. J of Immunol, 2013
4. Zhao Y, et. al. Oncotarget, 2017
5. Klussimeier A, et al. Front. Immunol, 2020
6. Kaiser, BK et al. Nature Letters 2007
7. Wang, X, et al. *Biochem Biophys Res Commun*. 2009
8. Whalen KA, Abstract 3506: Cancer Research. 2022; 82:3506-350