Abstract # 3506

CLN-619, a clinical-stage MICA/MICB-specific IgG1 antibody, restores the MICA/MICB-NKG2D axis to promote NK-mediated tumor cell lysis

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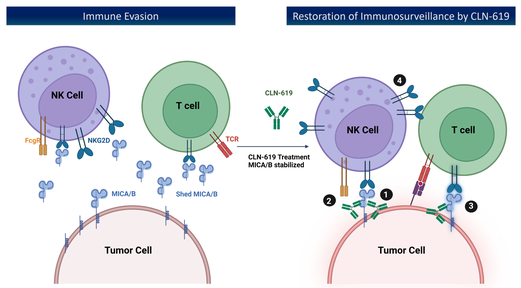
Background

- 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 the tumor.[3]
- High concentrations of shed MICA have been observed in sera from patients across multiple tumor types and 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 human MICA and MICB and is cross-reactive to the NHP orthologs.
- CLN-619 prevents the proteolytic release of MICA/MICB thereby exposing tumor cells for immune destruction through both NKG2D-mediated and antibody-dependent cell-mediated cytotoxicity (ADCC).
- 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



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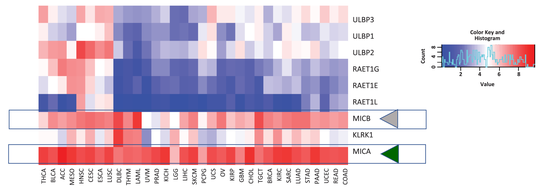
1. Prevention of MICA/MICB shedding, restoring NKG2D engagement of tumor cells
2. Antibody-dependent cellular cytotoxicity (ADCC)
3. Enhancement of binding of MICA to NKG2D
4. Reduction in decoy of NKG2D by shed MICA/B, avoiding NKG2D downmodulation/ decreased functionality

Results

Figure 2: MICA and MICB Are Broadly Expressed in Human Cancers

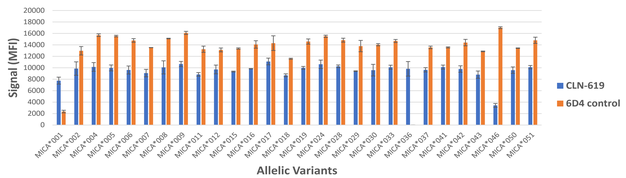


MICA/B are the most highly and most frequently expressed NKG2D ligands across 32 different tumor types (TCGA)

Figure 3: CLN-619 Binds with High Affinity to MICA and MICB Allelic Variants

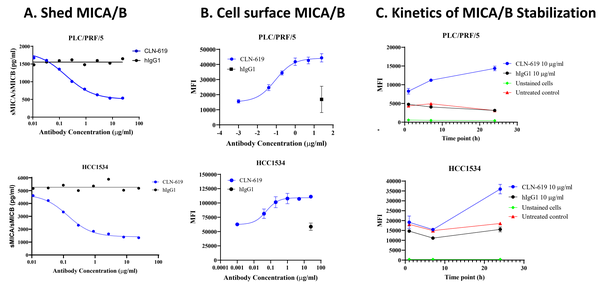
Allele	Allelic Frequency	K_D (nM)
MICA*001	0.7%	1.63 ± 0.12
MICA*002	11.7%	1.54 ± 0.12
MICA*004	6.5%	2.04 ± 0.12
MICA*008	42.8%	0.77 ± 0.17
MICB*004	21.7%	11.37 ± 1.36

Binding affinity of CLN-619 to human MICA and MICB proteins by Octet (N=3).



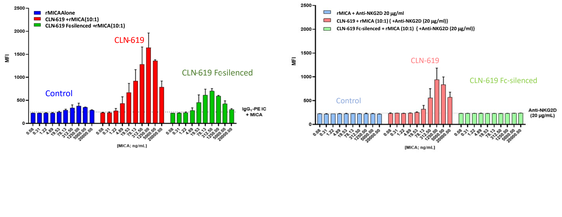
Luminex-based binding assay comprised of beads coated with recombinant human MICA ECD proteins representing 28 of the most prevalent MICA alleles N=2. The MICA/MICB specific antibody, 6D4, was previously demonstrated to have relatively broad reactivity in the Luminex assay and was used as a positive control.[6]

Figure 4: CLN-619 Inhibits MICA/MICB Shedding



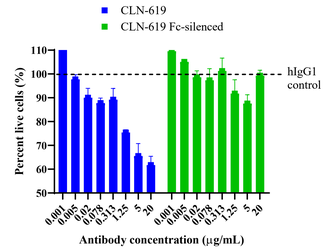
A–B) Cells were incubated with antibodies for 24 hours, N=2. A) MICA/MICB was measured in the cell supernatant by ELISA. B) MICA/MICB cell surface expression was measured by flow cytometry using the 6D4 antibody. C) Cells were incubated for indicated time points with 10 ug/ml of antibody, and MICA/MICB cell surface expression was measured by flow cytometry using the 6D4 antibody, N=2.

Figure 5: CLN-619 Enhances Binding of MICA to NKG2D



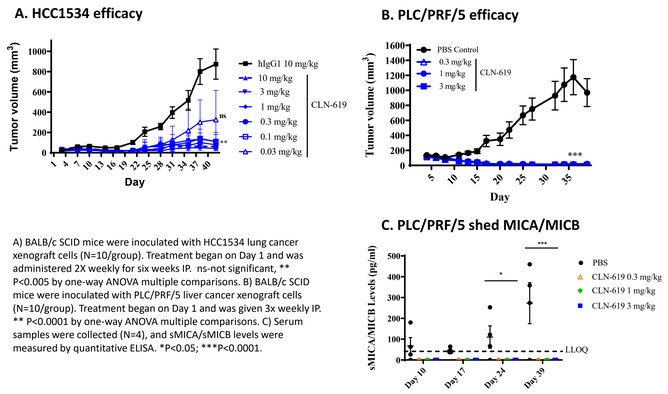
MICA binding to NKG2D expressing NK cells by flow cytometry. CLN-619 antibodies were pre-incubated with recombinant MICA (rMICA) followed by incubation with NK cells. NK cells were either A) untreated or B) pre-blocked with anti-NKG2D antibody. (Mean ± SD, N=2). Control samples were not treated with CLN-619 antibodies.

Figure 6: CLN-619 Induces Immune-Mediated Tumor Cell Killing *In Vitro*



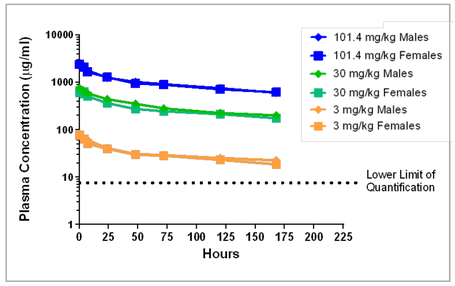
CLN-619, CLN-619 Fc-silenced or hIgG1 control antibodies were pre-incubated with HCC1534 cells (24 hr), and then co-incubated with human donor PBMC for 12 hrs (E:T ratio of 40:1). Cytotoxicity of HCC1534 was measured with Cytolight rapid red dye® and Incucyte® imager. Data representative of two donors.

Figure 7: *In Vivo* Efficacy of CLN-619 in Human Xenograft Models



A) BALB/c SCID mice were inoculated with HCC1534 lung cancer xenograft cells (N=10/group). Treatment began on Day 1 and was administered 2X weekly for six weeks IP. ns-not significant, ** P<0.005 by one-way ANOVA multiple comparisons. B) BALB/c SCID mice were inoculated with PLC/PRF/5 liver cancer xenograft cells (N=10/group). Treatment began on Day 1 and was given 3x weekly IP. ** P<0.0001 by one-way ANOVA multiple comparisons. C) Serum samples were collected (N=4), and sMICA/sMICB levels were measured by quantitative ELISA. *P<0.05; ***P<0.0001.

Figure 8: PK and Safety of CLN-619 in NHPs



- GLP toxicology study was performed in cynomolgus monkeys
- Monkeys were injected with CLN-619 IV at 3, 30 and 101.4 mg/kg, Q1W for 5 weeks
- CLN-619 exposures were approximately dose proportional
- 12d half-life in monkeys; 27d half-life estimated in humans
- No CLN-619-related findings noted, including no effects on cytokine levels or WBCs
- The NOAEL of CLN-619 is equivalent to the HNSTD, 101.4 mg/kg/week

Conclusions

- CLN-619 exhibited high affinity binding to all common allelic variants of MICA and the canonical allelic variant of MICB.
- CLN-619 prevents proteolytic release of MICA/MICB from cells resulting in increased cell surface expression of MICA/MICB, peaking at 24 hours *in vitro*.
- CLN-619 enhances the binding between recombinant MICA and NKG2D on NK cells. This activity was attributed to both FcγR engagement on NK cells as well as an intrinsic enhancement of binding of MICA to NKG2D.
- CLN-619 treatment of MICA-expressing tumor cells resulted in immune-mediated cell killing *in vitro* and was dependent upon a functional Fc-domain.
- In the pivotal GLP toxicology study in monkeys, no CLN-619-related findings were noted, and the NOAEL was defined as the highest dose administered, i.e., 101.4 mg/kg/week.
- CLN-619 exhibited potent *in vivo* anti-tumor activity in mice bearing MICA/MICB-expressing human tumor xenografts and reduced levels of shed MICA/sMICB in sera from CLN-619 treated animals.
- A Phase 1 clinical trial with CLN-619 as monotherapy and in combination with pembrolizumab is in progress.

References
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