CLN-619, a clinical-stage MICA/B-specific IgG1 antibody which restores the MICA/B-NKG2D axis requires Fc function for potent anti-tumor activity

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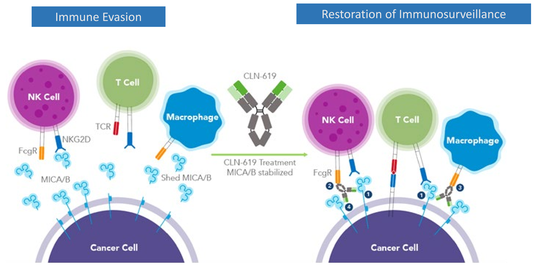
Background

- 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 non-human primate orthologs.
- CLN-619 prevents the proteolytic release of MICA/MICB from the tumor cell surface thereby exposing tumor cells for immune destruction through NKG2D-mediated and Fc- dependent mechanisms (ADCC & 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).

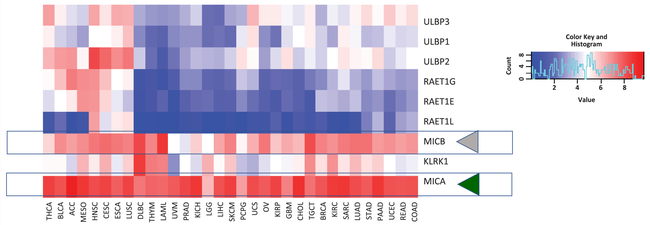
Figure 1: CLN-619 Multiple Modes of Action



1. Prevention of MICA/MICB shedding, restoring recognition of tumor cells by immune cells
2. Antibody-dependent cellular cytotoxicity (ADCC)
3. Antibody-dependent cellular phagocytosis (ADCP)
4. Enhanced binding of MICA to NKG2D

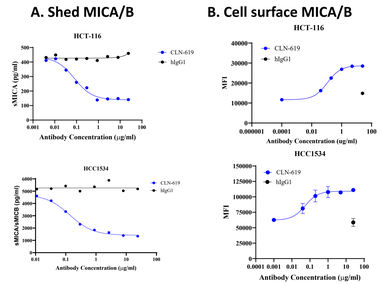
Results

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



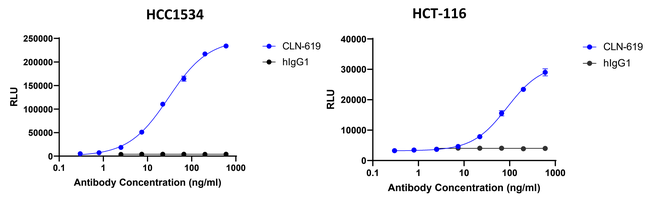
MICA/MICB are the most highly and frequently expressed NKG2D ligands across 32 tumor types (TCGA)

Figure 3: CLN-619 Inhibits MICA/MICB Shedding



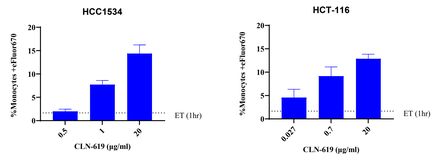
A-B) Cells were incubated with antibodies for 24 hours, N=2. A) Shed MICA/MICB was measured in the cell supernatant by ELISA. B) MICA/MICB cell surface expression was measured by flow cytometry.

Figure 4: CLN-619 Activates CD16/FcγRIII Signaling Leading to ADCC



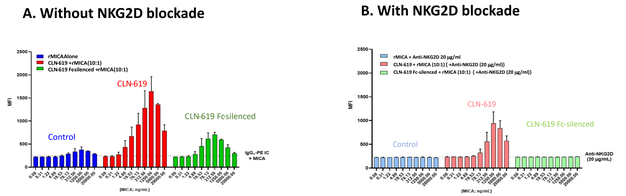
Jurkat cells expressing human FcγRIIIaV and an NFAT-luciferase reporter (Promega) were co-cultured with MICA/MICB-expressing tumor cells and incubated with hIgG1 or CLN-619. Luciferase activity was measured at 6 hours.
CLN-619 activates CD16/FcgammaRIIIaV signaling leading to ADCC

Figure 5: CLN-619 Mediates ADCP



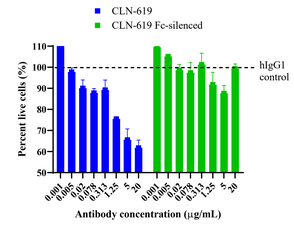
Uptake of target cells by effector cells was measured by flow cytometry. eFluor450-labeled effector donor cells were co-incubated with eFluor670-labeled target cells (E:T ratio of 1:1) in the presence of CLN-619 or CLN-619 Fc-silenced antibodies for 1 hour. The percent of viable, double stained CD14+ cells was measured by flow cytometry.

Figure 6: CLN-619 Enhances Binding of MICA to NKG2D



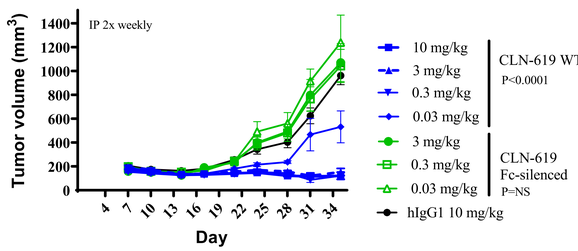
MICA binding to NKG2D expressing NK cells was measured by flow cytometry. CLN-619 was pre-incubated with recombinant MICA (rMICA) followed by incubation with NK cells. NK cells were either A) untreated or B) pre-blocked with anti-NKG2D antibody. (Mean ± SD, N=2). Control samples were not treated with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

Figure 7: CLN-619 Induction of Immune-Mediated Tumor Cell Killing *In Vitro* Depends Upon a Functional Fc-Domain



CLN-619, CLN-619 Fc-silenced or hIgG1 control antibodies were pre-incubated with labeled HCC1534 cells (24 hours), and then co-incubated with human donor PBMC for 12 hours (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 8: Efficacy of CLN-619 Depends Upon a Functional Fc-Domain



BALB/c SCID mice were inoculated with HCC1534 human lung cancer cells (N=10/group). Mice were treated 2X weekly IP with CLN-619 or CLN-619 Fc silenced at a range of doses. Statistics represent one-way ANOVA multiple comparisons. NS-not significant.

Conclusions

- CLN-619 prevents proteolytic release of membrane bound MICA/MICB from tumor cells resulting in increased cell surface expression of MICA/MICB.
- CLN-619 elicits both ADCC and ADCP in the presence of MICA/MICB-expressing cells in a dose dependent fashion.
- CLN-619 enhances the binding of recombinant MICA to 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/MICB-expressing tumor cells resulted in immune-mediated cell killing *in vitro* and was dependent upon a functional Fc-domain.
- CLN-619 exhibited potent, Fc-dependent, *in vivo* anti-tumor activity in mice bearing MICA/MICB-expressing human tumor xenografts

References
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