POSTER #194

Characterization of the Pharmacodynamic Activity of CLN-619, an Anti-MICA/B Monoclonal Antibody, in Cancer Patients from an Ongoing Phase 1 trial

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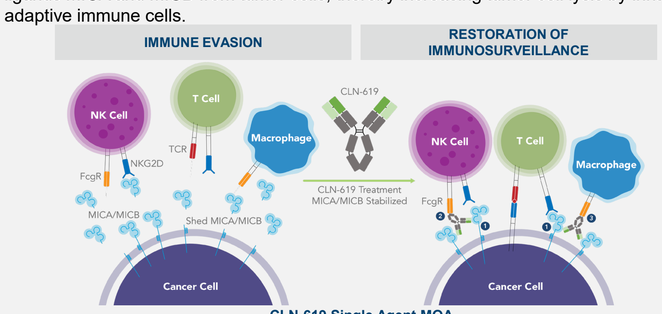
CLN-619 Engages Both Innate & Adaptive Immune Cells

CLN-619 is a human IgG1 antibody that binds to and prevents proteolytic cleavage of NKG2D ligands MICA and MICB from tumor cells, thereby increasing tumor cell lysis by innate and adaptive immune cells.



CLN-619 Single Agent MOA

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)

CLN-619-001 Phase 1 Study (NCT05117476)

- CLN-619-001 is first-in-human dose escalation and dose expansion study of CLN-619 administered alone (Module A) or in combination with pembrolizumab (Module B) in patients with advanced solid tumors.
- Monotherapy efficacy has been observed, including 3 confirmed responses at doses >= 3 mg/kg (ASCO Annual Meeting 2023 Abstract #2532)

Objective Response Summary

Dose Level	Tumor Type	Prior Therapy	Response Detail
3 mg/kg	**Parotid** (mucoepidermoid)	2 prior therapies Prior anti-PD1 (PR x 30 months)	Confirmed CR C4D1 **ongoing**
	Endometrial (serous)	5 prior therapies Prior anti-PD1 + lenvantinib (SD x 9 months)	PR at C4D1 **ongoing**
10 mg/kg	**Endometrial** (endometrioid)	3 prior therapies No prior anti-PD1	SD at C4D1 PR at C7D1 **ongoing**

- Pharmacodynamic activity associated with CLN-619 monotherapy is reported here

Biomarker Plan

Pharmacodynamic (PD) and mechanism of action (MoA) analysis for the CLN-619-001 Phase I study aimed at demonstrating PD effect of CLN-619 in the periphery and in the tumor microenvironment

Pharmacodynamic (PD) Biomarkers/ Mechanism of Action (MoA) | **Exploratory Predictive Biomarkers**

Peripheral PD/MoA

- **ELISA** (sMICA)
- **Flow Cytometry** (Immunophenotyping)
- **Luminex** (46 cyto-/chemokine panel)
- **ctDNA**** (Targeted NGS panel)

**Expansion cohorts

Intra-tumoral PD/MoA

- **IHC** (MICA/B/ & NKG2D)
- **Multiplex IF** (NK and T cell infiltration)
- **WES/ RNASeq** (Immune gene expression)

Paired tumor biopsy analysis for PD; Baseline (archival/fresh) for predictive analysis

Genotyping

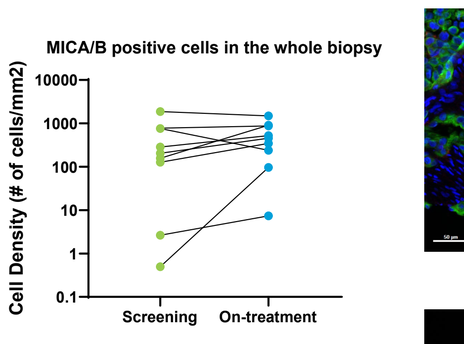
- **NGS typing** (MICA/B alleles)
- **Real-time PCR** NKG2D haplotype FCgRIIIA SNP (ADCC)

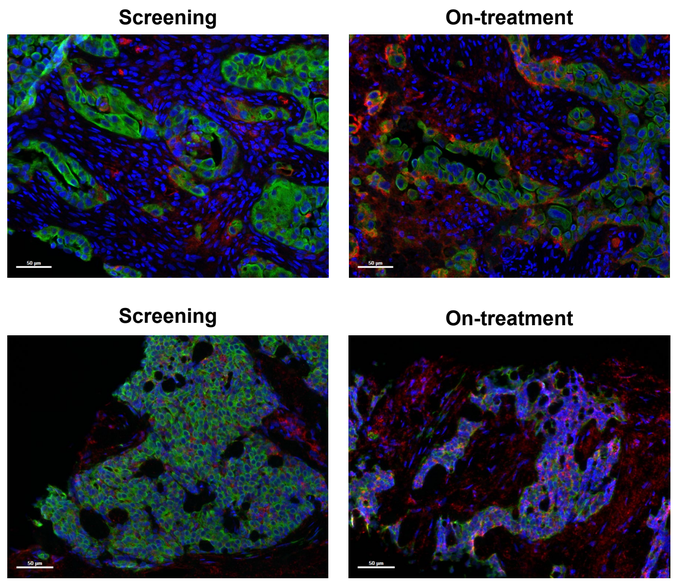
Whole blood at baseline

Intratumoral MICA/B Expression Increased On-treatment

Intratumoral MICA/B Expression Increased On-treatment with CLN-619: Multiplex IF

Increased MICA/B-positive staining in the majority of paired biopsies On-treatment (Cycle 2 Day 8) as compared to baseline (Screening)



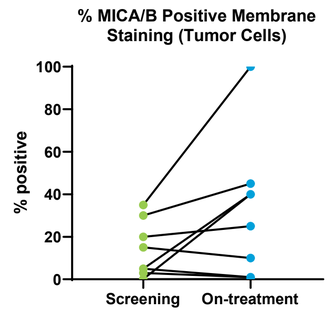


MICA/B expression levels was analyzed in 9 paired biopsies by MultiplexIF and quantified by the Image Analysis algorithm (Flagship Biosciences) Scale bar = 50µm

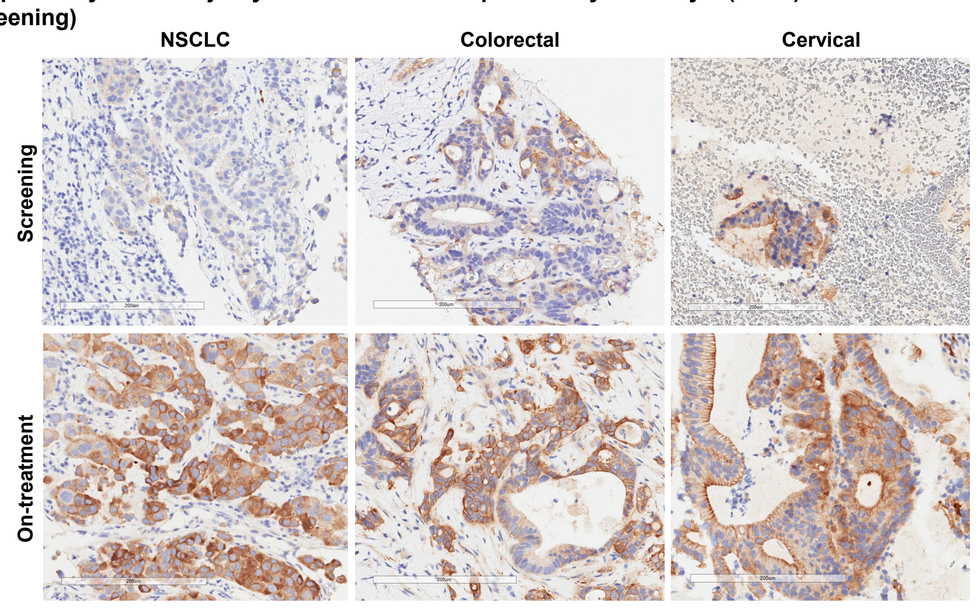
DAPI
MICA/B
PanCK

Cell Surface MICA/B Localization Increased in Tumor Biopsies On-treatment with CLN-619: IHC

Increased MICA/B membrane positivity in the majority of on-treatment biopsies at Cycle 2 Day 8 (C2D8) as compared to baseline (Screening)



MICA/B membrane localization was analyzed in 9 paired biopsies by IHC and quantified by a pathologist as % membrane staining positive cells. Scale bar = 200µm



Responding Endometrial Tumors are Microsatellite-Stable (MSS) and Have Low TMB and Low Neoantigen Presentation Index

Case Study 1: Endometrial (Endometrioid) Cancer - confirmed PR after 3 cycles of CLN-619

- 3 prior lines of therapy, no prior checkpoint inhibitor
- Archival specimen (~2 years prior to first dose CLN-619)

RNASeq/WES and genotyping results:

TMB NSN/Mb	NEOPS*	MSI Status**	NK cells ssGSEA score	T Cells - CD4+ ssGSEA score	T Cells - CD8+ ssGSEA score	T Cells - Tregs ssGSEA score	FCgR3A Genotyping§	NKG2D haplotype§§
2.89	2.58	MSS	-6,934	-1,507	-4,840	626	Heterozygous (V/F)	LNK1/LNK1

* NEOPS – neoantigen presentation score; ** MSI Status is defined by the Bethesda Consensus Panel; § FCgR3A V/F SNP confers intermediate affinity of the FC region of the antibody to FCgR; §§ LNK1/LNK1 homozygous NKG2D is associated with lower NK-cell activation (2).

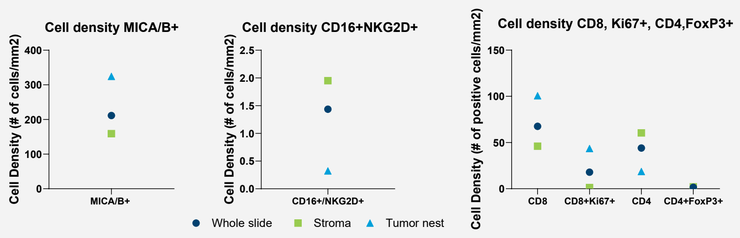
DAPI CD4 FoxP3 | DAPI CD8 Ki67 | DAPI PDL1

DAPI MICA/B | DAPI MICA/B PanCK | DAPI NKG2D CD16

MultiplexIF 6-plex panels were used for staining; expression of various markers was quantified by the Image Analysis algorithm (Flagship Biosciences). Scale bar = 50µm

Multiplex IF Image Analysis results:

- Low CD4 (60-20 cells/mm2) and intermediate CD8 T-cell infiltration (100-40 cells/mm2)
- Low PDL1 (3 positive cells/mm2)
- Low NK-cell infiltration
- MICA/B staining - high positivity



Case Study 2: Endometrial (Serous) Cancer – confirmed PR after 6 cycles

- Five prior lines of therapy, including pembrolizumab + lenvantinib (best response SD, last dose ~2 months prior to starting CLN-619)
- A fresh pre-treatment biopsy was collected at screening

RNASeq/WES and genotyping results:

TMB NSN/Mb	NEOPS	MSI Status	NK cells ssGSEA score	T Cells - CD4+ ssGSEA score	T Cells - CD8+ ssGSEA score	T Cells - Tregs ssGSEA score	FCgR3A Genotyping	NKG2D haplotype
0.17	0.14	MSS	-4,044	-973	4,993	1,561	Homozygous (F/F)	LNK1/LNK1

* NEOPS – neoantigen presentation score; ** MSI Status defined by the Bethesda Consensus Panel;
§ FCgR3A F/F SNP confers low affinity of the FC region of the antibody to FCgR; §§ LNK1/LNK1 homozygous NKG2D is associated with lower NK-cell activation (2).

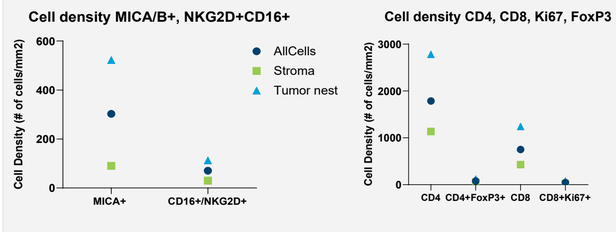
DAPI CD4 FoxP3 | DAPI CD8 Ki67 | DAPI PDL1

DAPI MICA/B | DAPI MICA/B PanCK | DAPI NKG2D CD16

MultiplexIF 6-plex panels were used for staining; expression of various markers was quantified by the Image Analysis algorithm (Flagship Biosciences). Scale bar = 50µm

Multiplex IF Image Analysis results:

- High CD4 (1000-3000 cells/mm2) and CD8 T-cell infiltration (300-1000 cells/mm2)
- High PDL1 (20 cells/mm2)
- Low NK-cell infiltration
- MICA/B staining positive



Tumor Microenvironment Analysis in Patients with Stable Disease Lasting ≥6 Months

Tumor Biopsies from Patients with Stable Disease Lasting ≥6 months

- Paired biopsies (screening and Cycle 2 Day 8) were obtained from patients (n =13) treated in the dose level extension cohorts at 3, 6, and 10 mg/kg dose levels.
- Paired biopsies were not available for any of the 3 responding patients.
- Paired biopsies were obtained from 3 patients otherwise meeting criteria for disease control (SD ≥ 6 months):

Tumor type	Dose
Cervical	3mg/kg
Adenoid Cystic H&N	3mg/kg
Mediastinal intimal sarcoma	10mg/kg

Low Tumor Mutation Burden (TMB) and Low Neoantigen Presentation Index in Tumors with Prolonged SD

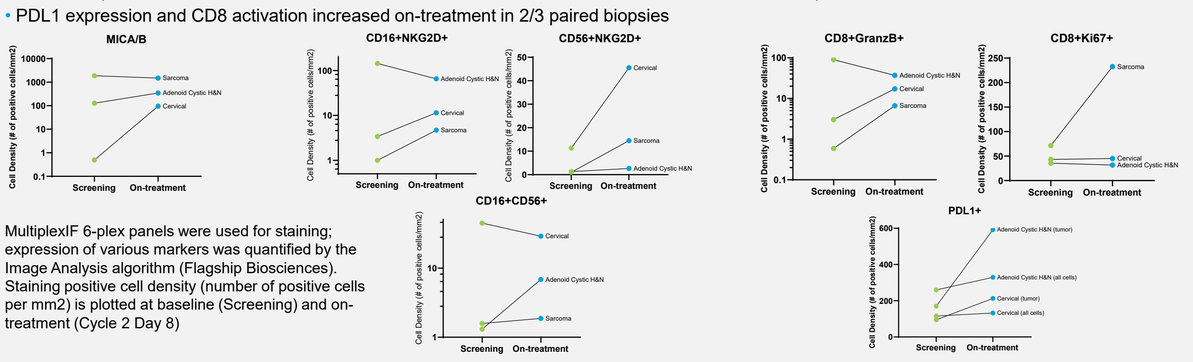
WES and RNASeq analysis demonstrated low tumor mutation burden (TMB) and low neoantigen expression index in tumors with prolonged SD:

Cancer type	Time of biopsy collection	TMB*** (NSN/Mb)	NEOPS**
Adenoid Cystic H&N	Screening	0.84	0.57
	On-treatment	0.88	0.66
Sarcoma	Screening	1.26	0.86
	On-treatment	1.49	1.58

* Sequencing data from the cervical sample is not available
** NEOPS – neoantigen presentation score
*** NSN – non-synonymous mutations

Increased MICA/B Expression and NK-cell Activation in In Paired Biopsies from Patients with Prolonged SD

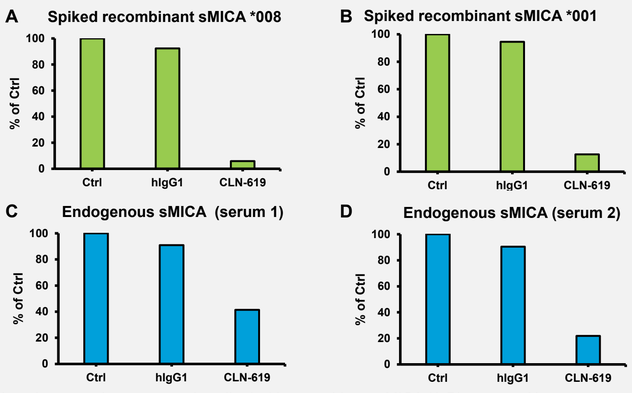
- MICA/B expression and NK-cell activation were increased on-treatment with CLN-619 in tumors from patients with SD of ≥6 months
- PDL1 expression and CD8 activation increased on-treatment in 2/3 paired biopsies



MultiplexIF 6-plex panels were used for staining; expression of various markers was quantified by the Image Analysis algorithm (Flagship Biosciences). Staining positive cell density (number of positive cells per mm2) is plotted at baseline (Screening) and on-treatment (Cycle 2 Day 8)

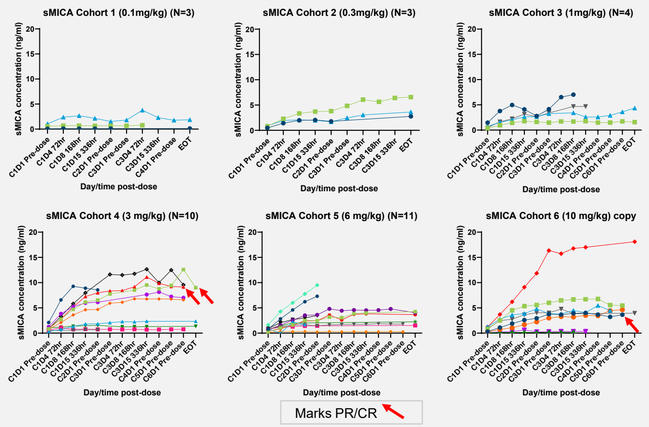
Soluble MICA in Patient Serum is a Pharmacodynamic Biomarker for CLN-619

CLN-619 Binds to sMICA in Human Serum



Negative control serum sample spiked with recombinant sMICA (A,B) or normal human serum (C,D) were pre-cleared of endogenous IgGs using Protein G Sepharose beads. Supernatant after beads clearance (Ctrl) was immunoprecipitated with hIgG1 or CLN-619 as indicated. sMICA remaining in the supernatant after immunoprecipitation was quantified by ELISA and plotted as percentage of Ctrl.

sMICA Levels Increase in Serum upon CLN-619 Treatment

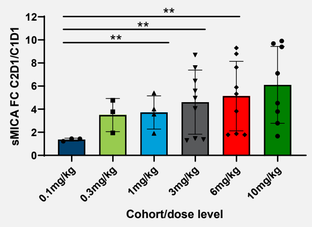


sMICA levels were measured by a qualified ELISA assay in human serum with anti-human MICA monoclonal antibody (clone AMO1(1)) as capture and anti-human monoclonal MICA/B antibody (clone BAMO1(1)) as detection.

sMICA Levels Are a Surrogate for Target Engagement by CLN-619

- **sMICA levels are a good measure of target engagement by CLN-619**, as evidenced by preliminary modeling of CLN-619 drug concentrations that predicted sMICA levels.
- **No sink effect has been observed**: CLN-619 is found in ~1000X excess in patient blood relative to sMICA levels, suggesting that only a small fraction of CLN-619 is bound to sMICA at any time.
- The small fraction of **CLN-619 bound to sMICA has not been observed to be clinically relevant**

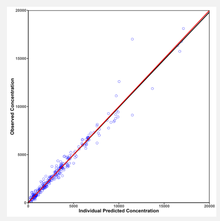
Fold change sMICA C2D1/C1D1



sMICA levels in patients' sera were measured by an ELISA assay and depicted as fold change (FC) of Cycle 2 Day 1 pre-dose (C2D1) over Cycle1 Day 1 (C1D1) pre-dose sMICA concentration

Preliminary PopPK Model Goodness of Fit Plot

- Predictions are similar to the observed data demonstrated by the closeness of the points to the line of unity.



Observed sMICA concentrations across dose levels vs concentrations predicted by the preliminary PopPK model

Conclusions

- **Intratumoral MICA/B expression is increased on-treatment with CLN-619 in paired tumor biopsies demonstrating the proof of biology**
  - Membrane localization of MICA/B is increased consistent with the proposed MoA
- sMICA is a Pharmacodynamic Biomarker for CLN-619
  - sMICA levels are increased on-treatment in a dose-dependent manner.
  - sMICA is a good correlate of target engagement.
- Objective responses and prolonged stable disease (≥ 6months) were observed in tumors with low TMB and low antigen presentation index:
  - Potential for CLN-619 to elicit response independent of proficient antigen presentation machinery
  - Consistent with the proposed multi-modal mechanisms of action of CLN-619

References:
1. Salih H.R., et al. J Immunol 2002.
2. Hayashi T., et al. Cancer Res. 2006.

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