Preclinical efficacy of CLEC-1 antagonist as novel myeloid immune checkpoint therapy for oncology

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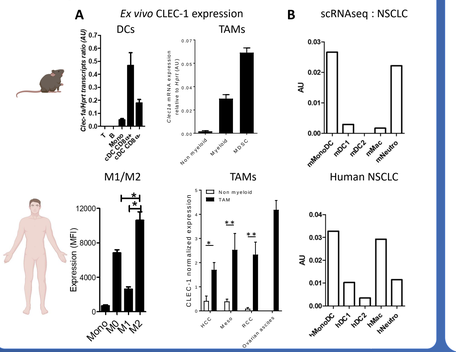
Abstract

C-type lectin receptors (CLRs) are powerful pattern recognition receptors shaping immune cell–mediated tissue damage by positively or negatively regulating myeloid cell functions and hence tumor elimination or evasion. We reported that the orphan CLR CLEC-1 expressed by dendritic cells (DCs) tempers T cells responses *in vivo* by limiting antigen cross-presentation by cDC1. Furthermore, we observed that CLEC-1 is highly expressed by myeloid cells purified from human tumor microenvironment, in particular tumor-associated macrophages. We found that CLEC-1 fusion protein, binds specifically to secondary necrotic healthy or tumor cells induced by chemotherapy, radiation (UV, X-ray) or culture stress conditions illustrating that **CLEC-1 ligand** is **inducible upon stress** and programmed cell death. Using newly developed anti-human CLEC-1 monoclonal antibodies (mAbs), we found that antagonist anti-CLEC-1 mAbs with the capacity to **block CLEC-1/CLEC-1 ligand interaction**, as opposed to non-antagonist CLEC-1 mAbs, **increase the phagocytosis** of CLEC-1 ligand-positive human tumor cells by human macrophages. Moreover, using *Clec1a* KO mice, we found that deficiency of CLEC-1 elicits **robust anti-tumor immune responses** and strong **modifications of the tumor microenvironment** and confirms for the first time this preclinical efficacy with recombinant **CLEC-1 blocking agents**.
These data illustrate that CLEC-1 inhibition represents a novel therapeutic target for immuno-oncology modifying T cell immune responses and tumor cell phagocytosis by macrophages.

1 CLEC-1 is expressed by tumor associated macrophages (TAM) and cross-presenting dendritic cells (cDC1)

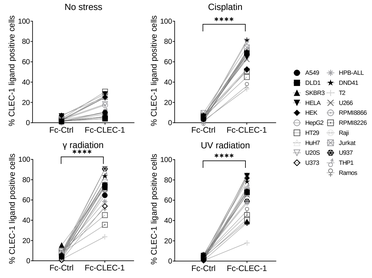
CLEC-1 expression in mouse and human immune cells and MΦ/DCs subsets from different solid tumor microenvironment context or models was analyzed by flow cytometry or RT-qPCR (**A**). *CLEC1A* expression was also characterized in mouse and human non-small cell lung carcinoma (NSCLC) tumor using single cell RNA sequencing public datasets (*Zilionis et al, Immunity 2019*) (**B**).



2 CLEC-1 ligand (CLEC-1L) is inducible under cell stress conditions and exposed by dying cells

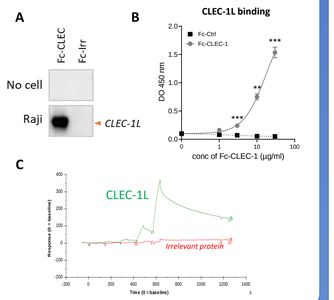
Ligand of CLEC-1 is detectable in danger-induced dying tumor cells

Human tumor cell lines were treated with 150mJ/cm² UV radiation, 50Gy γ−radiation, or 20µM of cisplatin for 24h and stained for flow cytometry analysis either by a Fc-Ctrl, or Fc-CLEC-1.
(NSCLC : non-small cells lung cancer A549; HCC : hepatocellular carcinoma Huh7, HepG2; OS : osteosarcoma U2OS; GB : glioblastoma U373; CRC : colorectal cancer HT-29, DLD1; triple negative breast cancer SK-BR3; ovarian cancer HeLa; human embryonic kidney HEK; T-ALL : T acute lymphoblastic leukemia HPB-ALL, DND41, Jurkat ; B-ALL Ramos, RPMI8866; myeloma U266, RPMI8226, lymphoma T2; acute myeloid leukemia THP-1, U937).



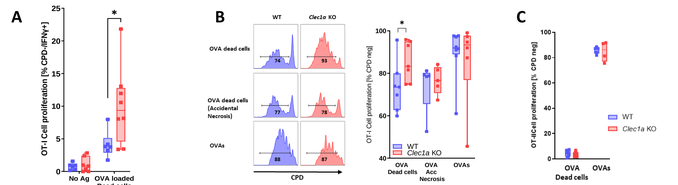
Identification of a CLEC-1 ligand in tumor cells

Human Raji tumor cell lysate was co-immunoprecipitated with hCLEC-1 recombinant protein and revealed by western blot (**A**). CLEC-1L was identified by MS/MS-SPECT and direct interaction confirmed by ELISA (**B**) and BIAcore (**C**).

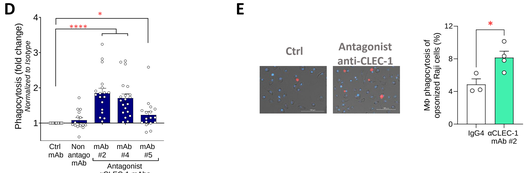


3 CLEC-1 impedes antigen cross-presentation and epithelial or hematopoietic tumor cell phagocytosis by human MΦ

DCs were generated from bone marrow of WT or *Clec1a* KO mice with 20ng/mL of GM-CSF, then incubated with MCA101-FcR OVA cells (expressing membranous OVA) treated either with UV (to induce CLEC-1L expression) and incubated or with OVA-specific CD8+ OT-1 cells in vitro (**A**) or with MCA101-FcR OVA cells freeze-thaw cycles (not expressing CLEC-1L) ; or soluble OVA protein which were concomitantly injected into *Clec1a* KO or WT mice with OVA-specific CD8+ OT-1 cells (**B**) or OVA-specific CD4+ OT-II cells (**C**) for *in vivo* experiment. OT-1 and OT-II proliferation was analyzed by flow cytometry.

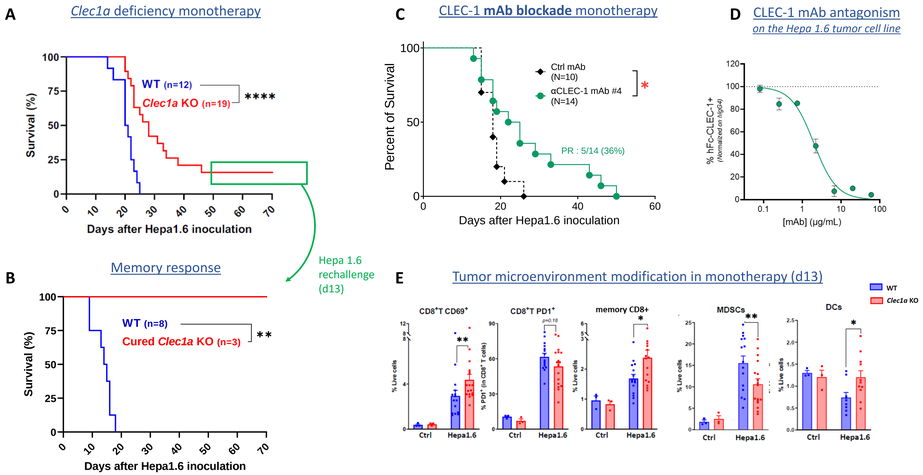


Human macrophages (MΦ) were generated from monocytes with M-CSF (100ng/mL) for 5 days, then polarized with IFNγ (70ng/mL) for two days to generate phagocytic MΦ. MΦ were cultured with the non-Hodgkin's lymphoma (Raji), at a 1:2 ratio + the anti-CD20 mAb (rituximab) at 10ng/mL providing the "Eat-me" signal, for 1 hour. Phagocytosis was analyzed by flow cytometry (**D**) or by microscopy (**E**) and normalized over the control antibody condition for each donor. * p<0.05; **** p<0.001 compared to isotype control.



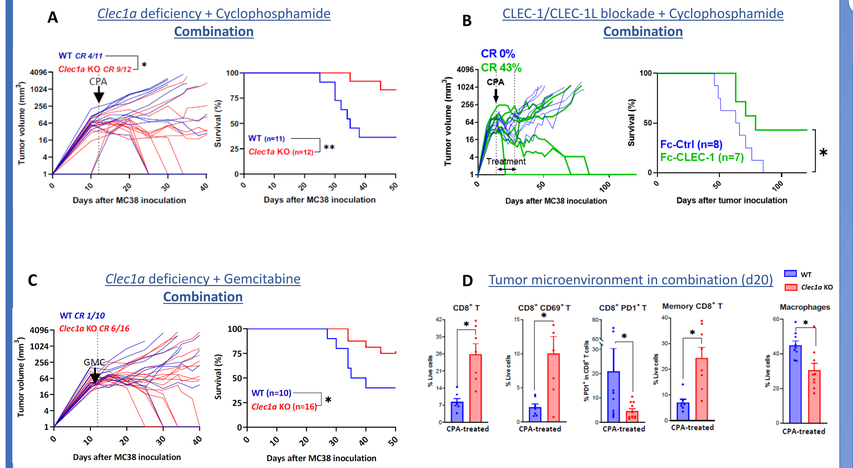
4 CLEC-1 deficiency or blockade impairs tumor growth of orthotopic solid tumors in monotherapy

Clec1a KO or WT mice received either 2.5x10⁶ Hepa 1.6 hepatocarcinoma cells in the portal vein (**A**), or in the spleen in CLEC-1 KO cured mice for tumor rechallenge (**B**). Anti-CLEC-1 antagonistic antibody was administered at 5mg/kg twice a week for 3 weeks starting to d4 after tumor inoculation in human CLEC1 knock-In mice (**C**). Tumor microenvironment (TME) analysis was conducted by flow cytometry on Percoll-sorted non parenchymal cells from tumor at d13 after tumor inoculation (**E**).



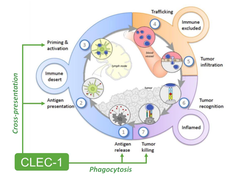
5 CLEC-1 deficiency or blockade improves anti-tumor chemotherapy response in solid tumors

Clec1a KO or WT mice subcutaneously received 1x10⁶ MC38 colorectal cancer cells. Chemotherapy (100mg/kg cyclophosphamide (**A-B**) or 50mg/kg gemcitabine (**C**)) was administered intraperitoneally (i.p.) once at d12. Mouse Fc-CLEC-1 recombinant protein was intraperitoneally injected twice a week for two weeks from d4 at 3mg/kg (**B**). Tumor microenvironment (TME) analysis was conducted by flow cytometry on Percoll-sorted non parenchymal cells from tumor at d20 after tumor inoculation (**D**).



Conclusion

- CLEC-1 is expressed by dendritic cells and tumor associated macrophages in human
- Identification of CLEC-1 Ligand in stressed tumor cells (UV, X-ray, Chemotherapy)
- CLEC-1/CLEC-1L interaction inhibits:
  - **T-cell cross-priming** by dendritic cells
  - Macrophages **tumor cell phagocytosis**
- CLEC-1 KO mouse:
  - Significant anti-tumor responses in monotherapy
  - Synergy with chemotherapy
  - Strong modification of TME (e.g. increased memory CD8 T cells)
- CLEC-1 antagonist mAbs or recombinant protein:
  - Promotes tumor cell phagocytosis by human Macrophages
  - Synergy with tumor-targeting Abs
  - Prolongs survival in HCC preclinical model and synergy with chemotherapy in CRC
- High interest in immune desert and to fight **Radiotherapy and Chemotherapy resistances**.



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