Blockade of the myeloid CLEC-1 checkpoint enhances antitumor responses and tumor antigen cross-presentation

Vanessa Gauttier[1,ǂ], Marion Drouin[1,ǂ], Isabelle Girault[1], Mylène Déramé[1], Stéphanie Neyton[1], Irène Baccelli[1], Javier Saenz[3], Emmanuel Merieau[3], Bérangère Evrard[3], Caroline Mary[1], Cécile Batty[1], Virginie Thépénier[1], Nicolas Poirier[1,ǂ], Elise Chiffoleau[3,ǂ]

[1]Ose Immunotherapeutics, Nantes, France; [2]CIFRE: OSE Immunotherapeutics, Nantes, France ; Nantes Université, Inserm, Centre de Recherche en Transplantation et Immunologie, UMR 1064, ITUN, F-44000 Nantes, France.
[3]Université de Nantes, [CHU Nantes], Inserm, Centre de Recherche en Transplantation et Immunologie, UMR 1064, ITUN, F-44000 Nantes, France; [ǂ]authors contribute equally to this work

AACR 2023
Poster #6358

Abstract

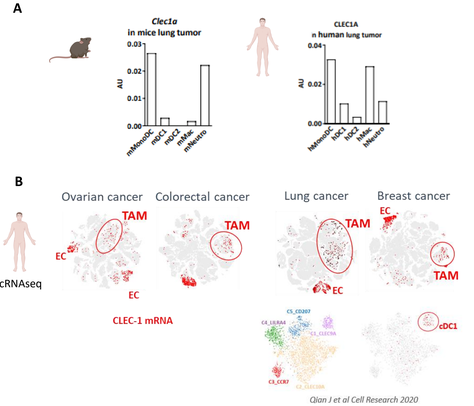
Myeloid cells represent the most abundant immune component of the tumor microenvironment, where they often assume immunosuppressive roles. We have identified CLEC-1, a member of the C-type lectin receptor (CLR) family as being expressed by myeloid cells, especially by conventional type one dendritic cells (cDC1), and by tumor-associated macrophages (TAM). Here, we investigated the effect of CLEC-1 blockade, either by genetic deletion or by antibody targeting, on myeloid function and anti-tumor response.

First, we observed that CLEC-1 genetic deletion significantly increases the survival of syngeneic tumor-bearing mice in the Hepa1.6 hepatocarcinoma, as well as in the AK-7 mesothelioma, orthotopic models. Moreover, the synergy with chemotherapy treatment in the MC38 model of colon adenocarcinoma significantly enhanced complete responses. Next, we generated anti-human CLEC-1 monoclonal antibodies (mAbs) as well as CLEC-1 humanized mice. We found that CLEC-1 targeting through mAb treatment was able to prolong mouse survival as efficiently as by CLEC-1 genetic deletion in MC38 and Hepa1.6 models. CLEC-1 blockade profoundly impacted the tumor microenvironment: increase of the frequency of invigorated dendritic cells (DCs) and macrophages, activated and memory T cells, while frequencies of immunosuppressive myeloid cells and PD1-expressing T cells largely decreased. Mechanistically, the in vivo phagocytosis of tumor cells (Hepa1.6 and MCA101) by macrophages was enhanced in CLEC-1 deficient animals compared to WT animals.

Altogether, our results demonstrate that CLEC-1 acts as a novel immune checkpoint in myeloid cells and highlight its high potential as a target for innovative immunotherapy in oncology.

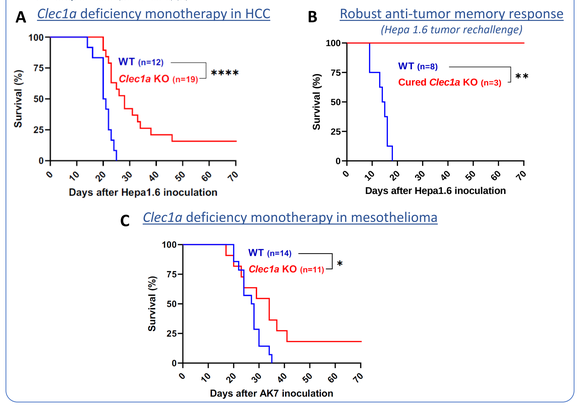
1 CLEC-1 is expressed by tumor associated macrophages (TAM) and cross-presenting dendritic cells (cDC1)

CLEC-1 expression in mouse and human immune cells and MΦ/DCs subsets from different solid tumor microenvironment context or models was analyzed by RT-qPCR (**A**). *CLEC1A* expression was also characterized in human cancer (lung, breast, ovarian, colon cancers) tumor using single cell RNA sequencing public datasets (TME Blueprint) (**B**).



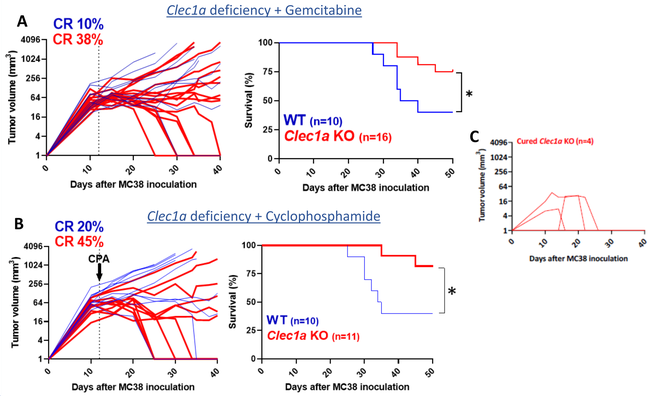
2 CLEC-1 deficiency impairs tumor growth of orthotopic solid tumors in monotherapy

Clec1a KO or WT mice received either 2.5x10⁶ Hepa 1.6 hepatocarcinoma cells in the portal vein (**A**), in the spleen in CLEC-1 KO cured mice for tumor rechallenge (**B**). or in the pleural cavity for the mesothelioma orthotopic model (3x10⁶ cells) (**C**)



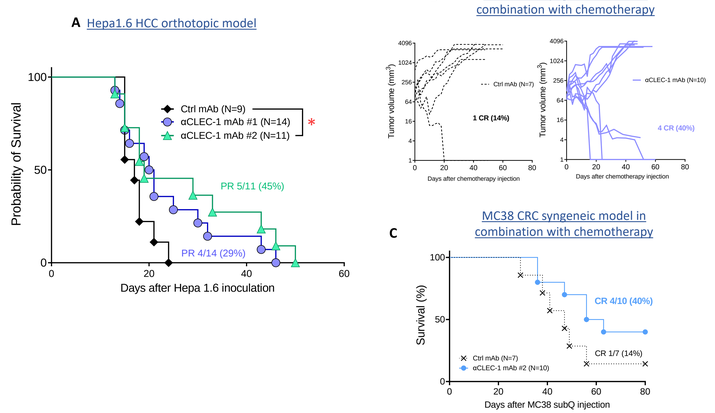
3 CLEC-1 deficiency synergizes with chemotherapy response in solid tumors

Clec1a KO or WT mice subcutaneously received 1x10⁶ MC38 colorectal cancer cells. Chemotherapy (50mg/kg gemcitabine (**A**) or 100mg/kg cyclophosphamide (**B**) was administered intraperitoneally (i.p.) once at d12. (C) Cured mice were rechallenged with 1x10⁶ MC38 on the other flank.



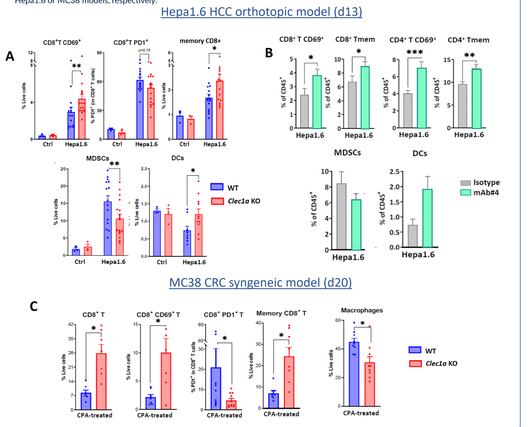
4 CLEC-1 blockade with antagonist mAbs induces robust anti-tumor responses

Humanized *Clec1a* knock-in mice received either 2.5x10⁶ Hepa 1.6 hepatocarcinoma cells in the portal vein (**A**) or 1x10⁶ MC38 colorectal cancer cells subcutaneously (**B**). Chemotherapy (100mg/kg cyclophosphamide) was administered intraperitoneally (i.p.) once at d12. Anti-CLEC-1 antagonistic antibodies were administered at 5mg/kg twice a week for 3 weeks starting to d4 after tumor inoculation.



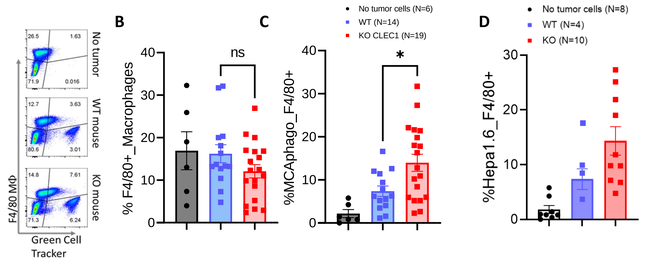
5 CLEC-1 deficiency or blockade deeply modifies the tumor microenvironment

Clec1a KO or WT mice (**A**) or humanized *Clec1a* knock-in mice (**B**) received 2.5x10⁵ Hepa 1.6 hepatocarcinoma cells in the portal vein or 1x10⁶ MC38 colorectal cancer cells subcutaneously (**C**). Chemotherapy (100mg/kg cyclophosphamide) was administered intraperitoneally (i.p.) once at d12. Anti-CLEC-1 antagonistic antibodies were administered at 5mg/kg twice a week starting to d4 after tumor inoculation. Immune cells were isolated for flow cytometry analysis from the tumor at d13 or d20 after tumor inoculation for Hepa1.6 or MC38 models, respectively.



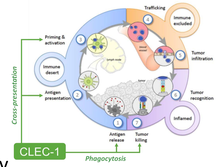
6 CLEC-1 inhibits tumor cell phagocytosis by mouse MΦ

MCA101 cells (fibrosarcoma) or Hepa 1.6 cells (hepatoma) were stained with CellTracker (ThermoFisher) and intraperitoneally injected (1.10⁶ cells/mouse) in WT or KO mice. Exudates were then collected after 1H after a 5mL injection of PBS-EDTA to harvest all cells. Macrophages were stained using an anti-F4/80 mAb and phagocytosis of tumor cells was evaluated in macrophages positive for the CellTracker Green. (A) Gating strategy. (B) Percentage of harvested macrophages in exudates. (C) Phagocytosis of MCA in macrophages (F4/80+). (D) Phagocytosis of Hepa 1.6 in macrophages (F4/80+).



Conclusion

- CLEC-1 is expressed by dendritic cells and tumor associated macrophages in human
- CLEC-1/CLEC-1L interaction inhibits macrophage **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)
  - Enhancement of *in vivo* phagocytosis of tumor cells
- CLEC-1 antagonist mAbs :
  - Prolongs survival in HCC preclinical model and synergizes with chemotherapy in CRC
  - Modifies the TME as observed in deficient mice
- High interest in immune desert and to fight **Radiotherapy and Chemotherapy resistances**.



Contact information : nicolas.poirier@ose-immuno.com ; vanessa.gauttier@ose-immuno.com