Antagonist antibodies inhibiting the binding of myeloid checkpoint CLEC-1 to novel endogenous ligands demonstrate high anti-tumor efficacies in humanized preclinical models

SITC 2023
Abstract #493

Irène Baccelli[1], Caroline Mary[1], Stéphanie Neyton[1], Mylène Deramé[1], Géraldine Teppaz[1], Emmanuelle Wilhelm[1], Marion Colonello[1], Kevin Biteau[1], Marion Drouin[1], Isabelle Girault[1], Vanessa Gauttier[1], Virginie Thépenier[1], Cécile Batty[1], Elise Chiffoleau[2], Nicolas Poirier[1].

[1] OSE Immunotherapeutics, 22 Boulevard Benoni Goullin, Nantes, France.

[2] Nantes Université, Inserm, Centre de Recherche en Transplantation et Immunologie Translationnelle, UMR 1064, ITUN, F-44000 Nantes, France

INTRODUCTION

CLEC-1 is a c-type lectin receptor belonging to the pattern recognition receptor family [Drouin, Saenz and Chiffoleau, Front Immunol., 2020]. It is a highly conserved protein, expressed by myeloid and endothelial cells in mice, non-human primates, and humans. Genetic deletion of CLEC-1 in mice does not lead to any developmental defect, but CLEC-1 deletion or CLEC-1 targeting through monoclonal antibody (mAb) treatment increases necrotic cell antigen cross-presentation by cDC1 dendritic cells, leading to enhanced T-cell activation and anti-tumor responses [Drouin et al., Science Adv., 2022].

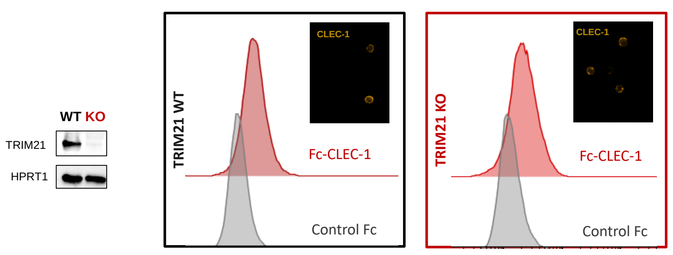
However, the signaling underlying this mechanism had not yet been fully understood: while a secreted endogenous ligand, Histidine-Rich Glycoprotein, HRG [Gao et al., iScience 2020] and an intracellular endogenous ligand, the intra-cellular E-3 ubiquitin ligase TRIM21 [Drouin et al., Science Adv. 2022] were previously identified, the existence of CLEC-1 endogenous cell surface ligands was unknown and the relative involvement of secreted, intracellular and cell surface CLEC-1 ligands in the inhibitory activity of CLEC-1 remained to be further investigated.

Here, we report the identification of novel endogenous ligands for CLEC-1, including some that are present at the cell surface. In addition, we investigate the relative importance of each of these ligands in the anti-tumor response exerted by different classes of anti-human CLEC-1 mAbs, thus shedding new light on the signaling underlying CLEC-1-mediated immune checkpoint activity.

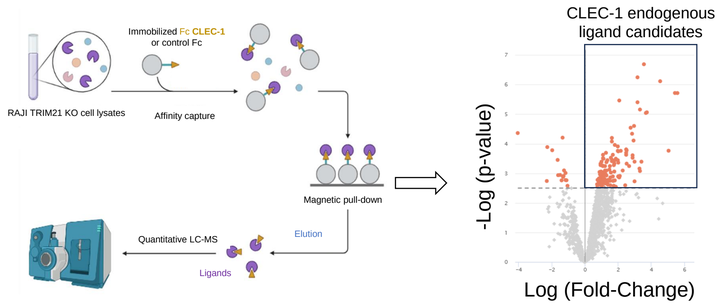
RESULTS

1. Identification of novel CLEC-1 endogenous ligands

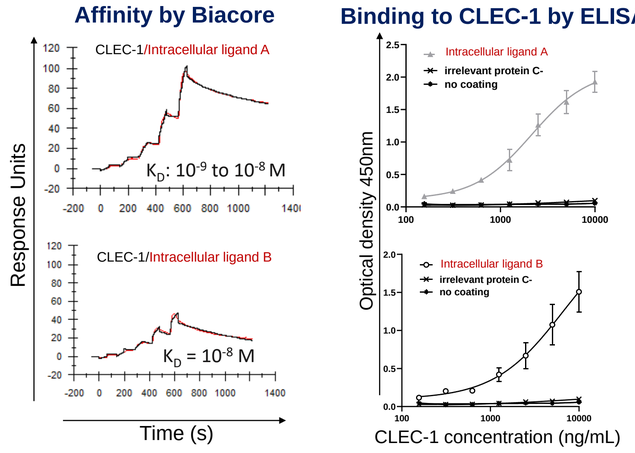
A) CLEC-1 binds to ligands different from TRIM21 on living cells



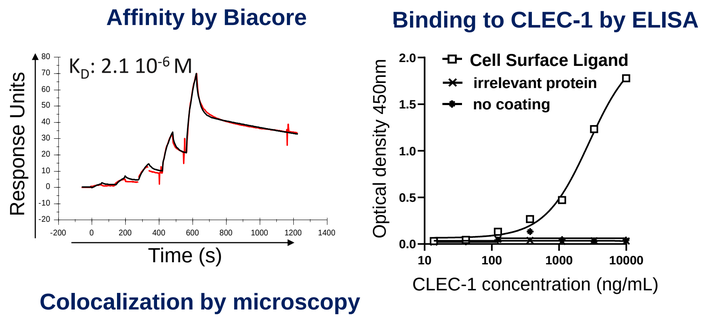
B) Novel CLEC-1 ligand identification strategy



C) Examples of intra-cellular CLEC-1 ligand validations

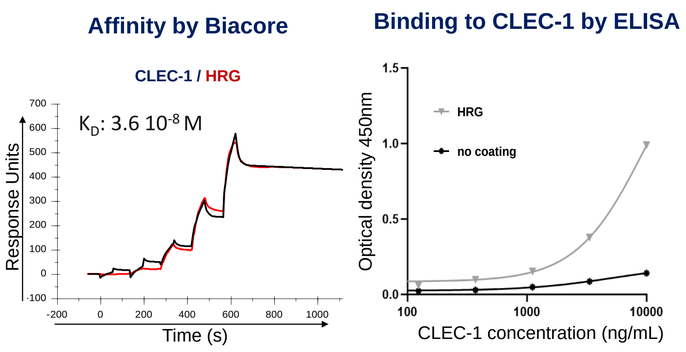


D) Example of cell surface CLEC-1 ligand validation

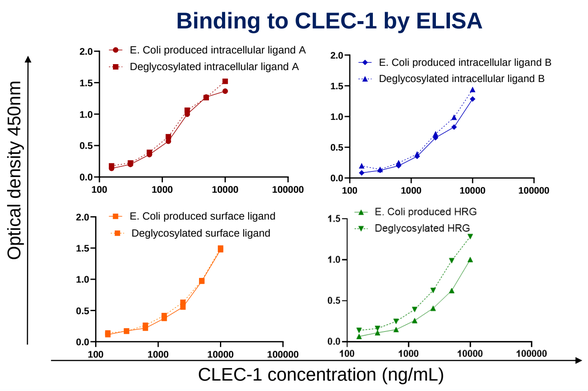


Colocalization by microscopy

E) Secreted CLEC-1 ligand (HRG) validation

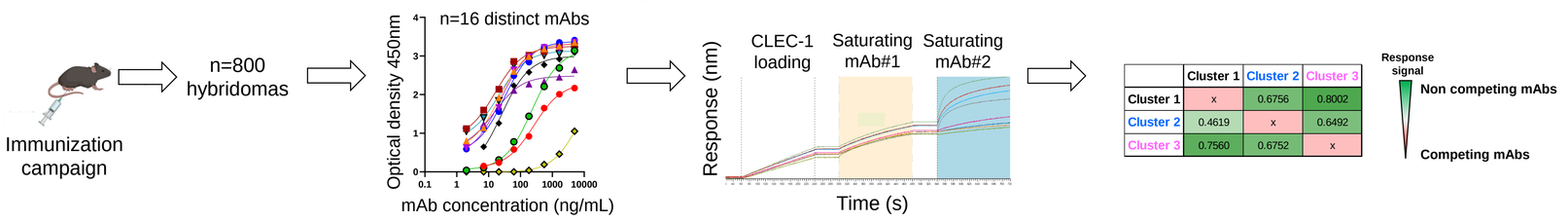


F) CLEC-1 interaction with its ligands is protein-specific



2. Generation of distinct clusters of anti-human CLEC-1 monoclonal antibodies (mAbs)

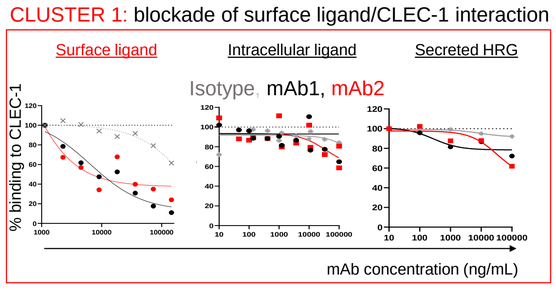
A) Identification of three main clusters of non-cross competing anti-human CLEC-1 mAbs



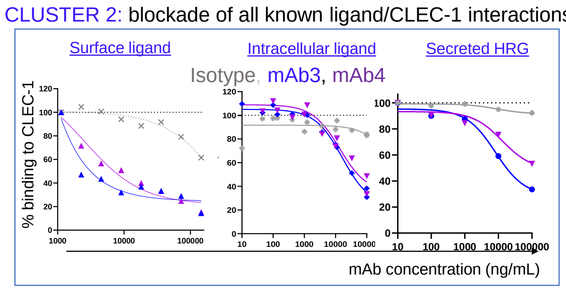
	Cluster 1	Cluster 2	Cluster 3
Cluster 1	x	0.6756	0.8002
Cluster 2	0.4619	x	0.6492
Cluster 3	0.7560	0.6752	x

B) Anti-human CLEC-1 mAb clusters show different competition profiles towards CLEC-1 ligand binding

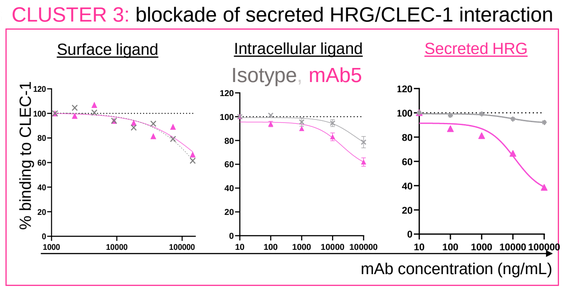
CLUSTER 1: blockade of surface ligand/CLEC-1 interaction



CLUSTER 2: blockade of all known ligand/CLEC-1 interactions



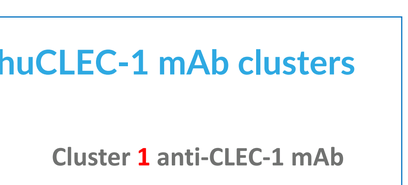
CLUSTER 3: blockade of secreted HRG/CLEC-1 interaction



3. *In vivo* monotherapy activity of anti-huCLEC-1 mAb clusters

CONCLUSIONS & OUTLOOK

- We report interactions between the immune checkpoint c-type lectin receptor CLEC-1 and several novel endogenous ligands, which we overall classify in 3 subcellular classes:
  1. intracellular ligands, such as TRIM21,
  2. a secreted ligand, Histidine-Rich Glycoprotein (HRG),
  3. and at least one ligand expressed at the cell surface.



- These molecular interactions are protein-specific as deglycosylation does not impact CLEC-1 binding to its endogenous ligands.
- Within a generated anti-human CLEC-1 antibody library, we identified 3 main clusters of non-cross-competing antibodies.
- These 3 clusters display distinct blocking activities towards the interaction of CLEC-1 with its intra-cellular / secreted / cell surface ligands.
- Preliminary *in vivo* interrogation in a syngeneic hepatocellular carcinoma mouse model indicates that all 3 classes of anti-CLEC-1 mAbs exert anti-tumoral activities.
- CLEC-1 interaction with its secreted ligand HRG may not fully underly the checkpoint activity of the receptor, as a blocking mAb specifically antagonizing this interaction induces limited anti-tumor response compared to other classes of mAbs.
- Further characterization of these anti-CLEC-1 mAb clusters will enable a better understanding of the molecular mechanisms underlying the anti-tumor activity of CLEC-1 targeting.

Contact information : nicolas.poirier@ose-immuno.com ; irene.baccelli@ose-immuno.com