Identification of Different Classes of Antagonist Monoclonal Antibodies Targeting the Myeloid Checkpoint CLEC-1 and Their Associated Anti-Tumoral In Vivo Efficacies in Humanized Preclinical Models

1Irène Baccelli*, 1Caroline Mary, 1Stéphanie Neyton, 1Mylène Deramé, 1Marion Drouin, 1Géraldine Teppaz, 1Emmanuelle Wilhelm, 1Isabelle Girault, 1Virginie Thepenier, 1Cécile Batty, 1Kevin Bitteu, 1Ariane Desselle, 1Marion Colonello, 1Julien Taurelle, 1Marie Malloci, 2Élise Chiffoleau, 1Nicolas Poirier. 1OSE Immunotherapeutics, Nantes, Loire-Atlantique, France; 2Nantes Université, Inserm, Centre de Recherche en Transplantation et Immunologie Translationnelle, UMR 1064, ITUN, Nantes, Loire Atlantique, France

Background The C-type lectin receptor CLEC-1 is a pattern recognition receptor expressed by endothelial and myeloid cells in mice, non-human primates, and humans. While genetic deletion of CLEC-1 in mice does not lead to any developmental defect, CLEC-1 deletion or CLEC-1 targeting using monoclonal antibodies increases damaged or necrotic cell antigen cross-presentation by cDC1 dendritic cells, as well as subsequent T-cell activation and anti-tumor response. However, the identification of CLEC-1 endogenous ligands and their relative involvement in the immune checkpoint activity of CLEC-1 remained to be fully investigated.

Methods Endogenous CLEC-1 ligand candidates were identified by affinity capture assays followed by LC/MS analysis. Ligand candidates were validated through protein-protein binding assays and Biacore affinity measurements. Through an immunization campaign, a library of anti-CLEC-1 monoclonal antibodies was generated and screened for CLEC-1 protein binding. Monoclonal antibodies were also assessed for their abilities to inhibit the binding of CLEC-1 to its newly identified endogenous ligands. Different classes of antagonist anti-CLEC-1 antibodies were thereby identified and subsequently evaluated for their anti-tumor efficacies in hepatocellular carcinoma (Hepa1.6) and colorectal cancer (MC38) preclinical models, using human CLEC-1 knock-in mice.

Results While we confirm CLEC-1 specific binding to the E3 ubiquitin ligase TRIM21 and to the secreted histidine rich glycoprotein (HRG), we also identify several novel intra-cellular and cell surface CLEC-1 ligands. We show that the binding of CLEC-1 to these newly identified ligands is protein-specific, as deglycosylation does not impair CLEC-1 binding to its ligands. Finally, we document the antitumoral activities of several classes of antagonist anti-CLEC-1 monoclonal antibodies: while blocking of CLEC-1 binding to its secreted ligand HRG moderately increases anti-tumor responses, inhibition of CLEC-1 binding to its cytoplasmic membrane ligands significantly impairs MC38 tumor growth (n=12, p=0.04) and increases the overall survival of Hepa1.6-bearing mice (n=12, p=0.002), as compared to corresponding isotype control treatment.

Conclusions Altogether, by shedding new light on the role of CLEC-1/CLEC-1 ligand interactions, our results further dissect the mechanism of action of the myeloid checkpoint CLEC-1 in its ability to impair anti-tumor immunity and support its use as a novel and highly promising target for cancer immunotherapy.

REFERENCES

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0493