Trifunctional NKp46/CD16a-NK cell engager targeting CD123 overcomes acute myeloid leukemia resistance to ADCC

#852

Laurent Gauthier[1†], Angela Virone-Oddos*[2†], Jochen Beninga[5], Benjamin Rossi[1], Céline Nicolazzi[2], Céline Amara[6], Audrey Blanchard-Alvarez[1], Nicolas Gourdin[1], Jacqueline Courta[7], Alexandra Basset[8], Franceline Guillot[1], Gwendoline Grondin[1], Helene Bonnevaux[2], Anne-Laure Bauchet[7], Ariane Morel[1], Yannis Morel[1], Marielle Chiron[2], Eric Vivier[1,3,4]

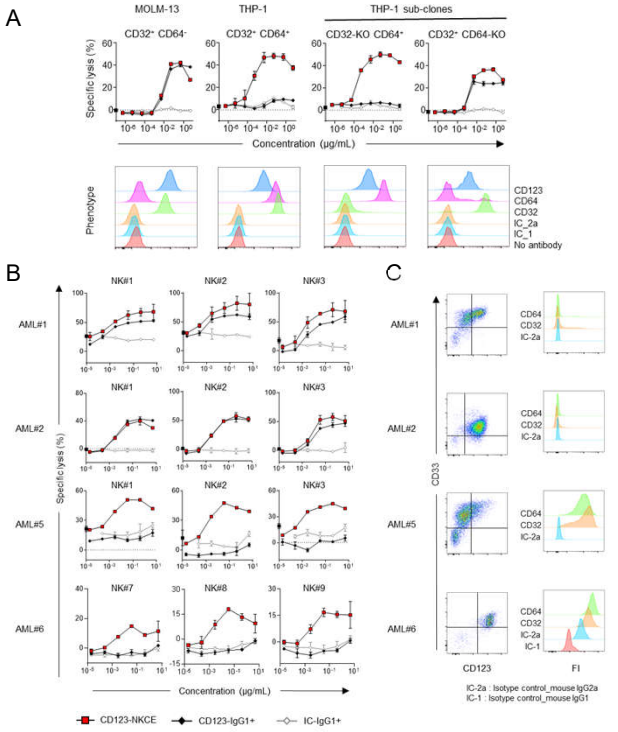
*[1]Innate Pharma, Marseille, France. [2]Sanofi Immuno-Oncology Research, Vitry sur-Seine, France.), [3]Aix Marseille University, CNRS, INSERM, CIML, Marseille, France. [4]Assistance Publique des Hôpitaux de Marseille, Hôpital de la Timone, Marseille-Immunopôle, Marseille, France.[5]Sanofi Large Molecules Research, Frankfurt, Germany.[6]Sanofi Drug Metabolism and Pharmacokinetics, Chilly Mazarin, France.[7]Sanofi TMED Biomarkers and Clinical Bioanalysis, Chilly Mazarin, France.[8]Sanofi Preclinical Safety, Chilly Mazarin, France.† :Co-first authors (equal contribution); * :Corresponding author (angela.virone-oddos@sanofi.com)*

Background

There is a clear need for targeted therapies to treat acute myeloid leukemia (AML), the most common acute leukemia in adults. CD123 (IL-3 receptor alpha chain) is an attractive target for AML treatment [1]. However, cytotoxic antibody targeting CD123 proved to be insufficiently effective in a combination setting in phase II/III clinical trials [2]. T-cell engagers targeting CD123 displayed some clinical efficacy but were often associated with cytokine release syndrome and neurotoxicity [3]. Interest in the use of NK cells for therapeutic interventions has increased in recent years, as a potential safer alternative to T cells. Several NK-cell activating receptors can be targeted to induce antitumor immunity. We previously reported the development of trifunctional NK-cell engagers (NKCEs) targeting a tumor antigen on cancer cells and co-engaging NKp46 and CD16a on NK cells [4]. We report here the design, characterization and preclinical development of a novel trifunctional NK cell engager (NKCE) targeting CD123 on AML cells and co-engaging NKp46 and CD16a on NK cells. We compared CD123-NKCE and a cytotoxic ADCC-enhanced antibody targeting CD123, in terms of antitumor activity *in vitro, ex vivo* and *in vivo*. Pharmacokinetic, pharmacodynamic and safety profiles of CD123-NKCE were evaluated in non-human primates (NHP).

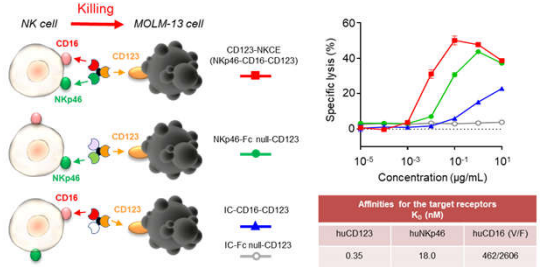
1 CD123-NKCE: strong cytotoxic activity against AML cells independently of high-affinity FcγRs expression on AML cells

The expression of high-affinity FcγR CD64 on AML cells inhibits the ADCC activity of ADCC-enhanced antibody (CD123-IgG1+) *in vitro* (A) and *ex vivo* (B-C) but does not affect CD123-NKCE killing activity.



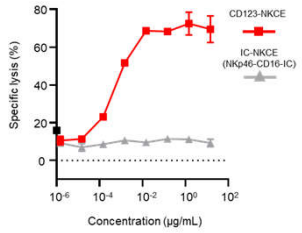
2 CD123-NKCE: strong potency against MOLM-13 AML cells *in vitro*

Co-engagement of NKp46 + CD16 for optimal NK cell activation



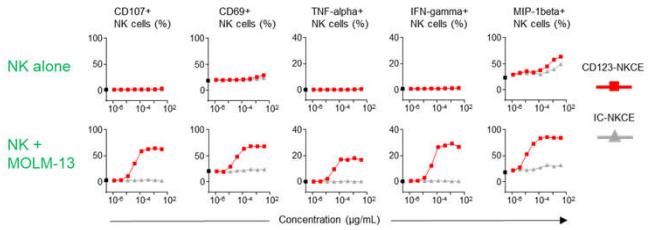
Affinities for the target receptors K_D (nM)		
huCD123	huNKp46	huCD16 (V/F)
0.35	18.0	462/2606

Cytotoxicity by NK cells against MOLM-13 AML cells

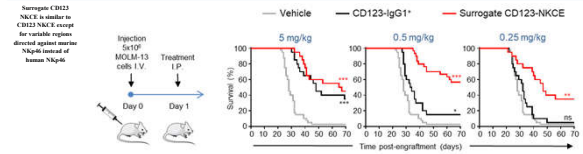


3 CD123-NKCE promotes NK cell activation, effector cytokine / chemokine production *in vitro*

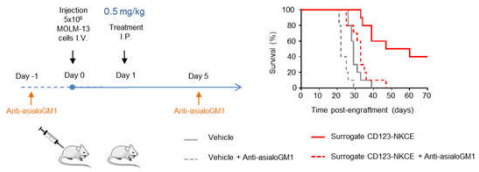
CD123-NKCE promotes NK cell activation (CD107a, CD69) and effector cytokines (TNFα & IFNγ) / chemokine MIP1β production *in vitro* only in presence of MOLM-13 target cells expressing CD123



4 CD123-NKCE: potent anti-tumor activity in mice model superior to anti-CD123 ADCC-enhanced IgG1 and NK cell-dependent



Depletion of NK cells *in vivo* abrogates CD123-NKCE-mediated anti-tumor effect



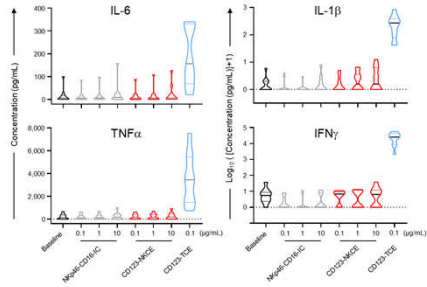
Acknowledgments

This work was done in collaboration with Magali Agnel, Alexandre Tang, Laurent Bassinet, Virginie Boisrobert-Dheilly.

References

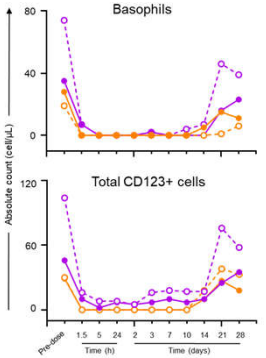
(1) Ehninger A, Kramer M, Rollig C, et al. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. Blood Cancer J. 2014; 4, e218.
(2) Montesinos P, Gail J Roboz GJ, et al. Safety and efficacy of talacotuzumab plus decitabine or decitabine alone in patients with acute myeloid leukemia not eligible for chemotherapy: results from a multicenter, randomized, phase 2/3 study. Leukemia. 2021;35(1):62-74.
(3) Uy GL, Aldoss I, Foster MC, et al. Flotetuzumab as salvage immunotherapy for refractory acute myeloid leukemia. Blood. 2021;137(6):751-762.
(4) Gauthier L, Morel A, Anceriz N, et al. Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity. Cell. 2019;177(7):1701-13.

5 CD123-NKCE leads to minimal cytokine release *in vitro* in human PBMC as compared to T-cell engager

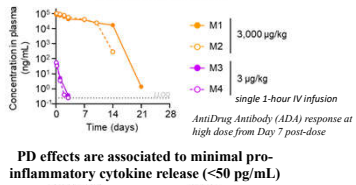


6 CD123-NKCE: PharmacoKinetics (PK) exposure and PharmacoDynamics (PD) effect in NHP is associated with very limited cytokine release and no clinical signs

Complete depletion of CD123+ immune cells in NHP at low & high doses

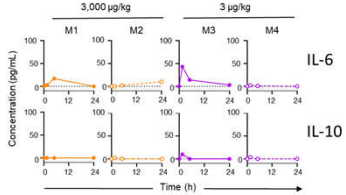


Target Mediated Drug Disposition (TMDD) observed at low dose



AntiDrug Antibody (ADA) response at high dose from Day 7 post-dose

PD effects are associated to minimal pro-inflammatory cytokine release (<50 pg/mL)



Of note, CD123-TCE at 3 µg/kg induces IL6 cytokine release in the 9,000 pg/ml range in NHP.

Conclusion

- The expression of the high affinity Fcγ receptor CD64 on patient-derived AML cells inhibited the ADCC activity of the antibody targeting CD123 *in vitro* and *ex vivo*, but not the antitumor activity of CD123-NKCE.
- CD123-NKCE led to potent antitumor activity against primary AML blasts and AML cell lines, promoted strong NK-cell activation and induced cytokine secretion only in the presence of AML target cells.
- Its antitumor activity in a mouse model was greater than that of the comparator antibody and dependent on the presence of NK cells.
- Moreover, CD123-NKCE generated strong and prolonged pharmacodynamic effects in NHP at very low doses, was well-tolerated up to 3 mg/kg and triggered only minor cytokine release.
- The data for activity, safety, pharmacokinetics, and pharmacodynamics provided here demonstrate the superiority of CD123-NKCE over the comparator cytotoxic antibody, in terms of antitumor activity *in vitro, ex vivo, in vivo*, and its favorable safety profile, as compared to T-cell therapies.
- These results demonstrate the efficacy of CD123-NKCE for controlling AML tumors *in vivo*, and provide consistent support for the clinical development of IPH6101 / SAR443579.