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Lirilumab Enhances Anti-Tumor Efficacy of Elotuzumab

Caroline Sola, PhD[1], Mathieu Bléry, PhD[1], Cécile Bonnafous, PhD[1], Elodie Bonnet[1], Nicolas Fuseri[1], Robert F Graziano, PhD[2], Yannis Morel, PhD[1] and Pascale André, PhD[1]

[1] Innate Pharma, Marseille, France; [2] Bristol-Myers Squibb, Princeton, NJ

Abstract

Tumor cells that express reduced levels of Major Histocompatibility Complex (MHC) class I molecules may be recognized and killed by Natural Killer cells (NK cells), through a process known as “missing self” recognition.

In humans, this is controlled by inhibitory receptors such as Killer Immunoglobulin-like Receptors (KIR) that recognize Human Leukocyte Antigen (HLA)-A, -B or –C. Engagement of KIR by HLA molecules results in inhibitory signaling that reduces NK cell-mediated natural killing and antibody dependent cellular cytotoxicity (ADCC). Hence, antibodies that block interactions between inhibitory KIR and their HLA ligands are being evaluated as an anti-cancer therapeutic strategy.

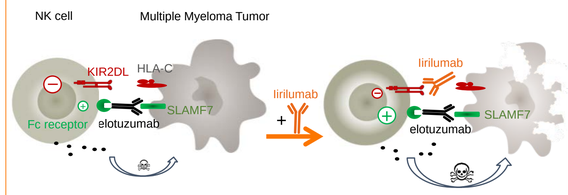
Lirilumab (BMS-986015 / IPH2102) is a fully human IgG_4 that blocks binding of inhibitory KIR to HLA-C, that is being developed for treating hematologic malignancies and solid tumors.

Elotuzumab (BMS901608 / HuLuc63) is a humanized IgG_1 anti-SLAMF7 (signaling lymphocyte activation molecule family member 7, CS-1) being developed for the treatment of Multiple Myeloma (MM). SLAMF7 is a cell surface glycoprotein highly expressed in myelomatous cells and only at low levels on normal cells.

NK cell-mediated ADCC is one of the main mechanisms of action of elotuzumab, but ADCC is negatively regulated by KIR checkpoint receptors. Thus a combination of lirilumab and elotuzumab has strong scientific rationale.

The aim of the present study was to assess whether lirilumab would enhance elotuzumab anti-MM activity *in vitro* with human peripheral blood NK cells and MM cell lines, and *in vivo* in a newly developed xenogenic mouse model.

Background



- ✓ NK cell inhibition by HLA-A/B/C
- ✓ elotuzumab-mediated ADCC negatively regulated by KIR checkpoint receptors
- ✓ Activation through KIR-HLA - blockade
- ✓ ADCC enhancement

Materials & Methods

- Two MM cell lines (OPM-2 and U266B1) were identified that express both MHC Class I and SLAMF7.

Figure 1: Expression of HLA-ABC and SLAMF7 on multiple myeloma cell lines U266B1 and OPM-2

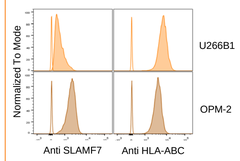


Fig. 1: FACS histograms of HLA-ABC and SLAMF7 on U266B1 and OPM-2 MM cell lines (Open histograms: isotype control, Filled histograms: indicated staining mAb).

- Functional *in vitro* assays were performed with peripheral blood mononuclear cells (PBMC) from healthy volunteers as effector cells (E/T ratio = 2.5).

Table 1: Characteristics of PBMC isolated from 12 healthy volunteers

Donor ID	CD16 typing	HLA-C typing	% NK in PBMC	% lirilumab positive NK cells
1	F/V	C1/C2	18%	76%
2	F/F	C1/C1	7%	37%
3	F/F	C1/C2	7%	52%
4	F/V	C1/C2	10%	42%
5	F/V	C2/C2	10%	33%
6	F/F	C1/C1	19%	52%
7	F/V	C1/C2	28%	59%
8	F/F	C1/C2	14%	68%
9	V/V	ND	8%	38%
10	V/V	C1/C1	4%	39%
11	V/V	ND	10%	51%
12	V/V	C1/C2	22%	43%
		Mean	**13%**	**49%**
		SD	**7%**	**13%**
		Median	**10%**	**47%**
		Range	**4-28%**	**33-76%**

- *In vivo* assessment of the therapeutic efficacy of lirilumab and elotuzumab was performed in transgenic mice expressing human KIR2DL3 (results reported in poster 4717) and as reported in this poster in a novel strain of double-transgenic mice we developed, expressing human KIR2DL3 as well as its ligand, HLA-cw3, on a $Rag1^{-/-}$ background (KIR-cw3-tg RAG mice). The OPM-2 MM cell line was subcutaneously engrafted in these mice and when high tumor volumes were reached (>100mm^3), mice were treated as indicated (10 mice per group).

Results

Figure 2: Lirilumab restores specifically KIR2D$^+$ NK cell activation towards multiple myeloma cell lines U266B1 and OPM-2 *in vitro,* in a dose dependent manner, and enhances elotuzumab-mediated ADCC

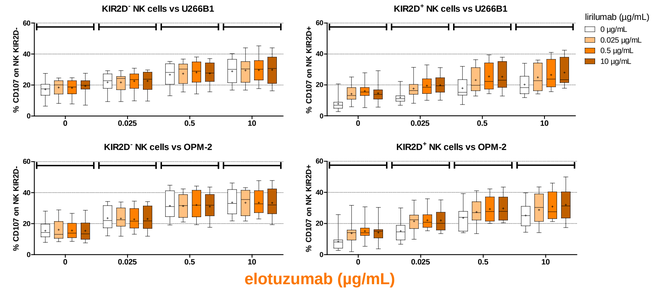


Fig. 2: CD107 mobilization on NK cells expressing KIR2D (KIR2D$^+$ NK cells, right column) targeted by lirilumab or on NK cells not expressing KIR2D (KIR2D$^-$ NK cells, left column). CD107 mobilization is measured on indicated NK cells, within PBMC incubated with U266B1 cells (top panel) or with OPM-2 cells (bottom panel) for 4h at 37°C. Box plots indicate the median with the second and third quartiles.

Whiskers indicate min. and max. values. + indicates the mean. N=12 healthy volunteers. Similar results were obtained by measuring intracellular cytokines (TNF-α, IFN-γ) contents (data not shown).

Figure 3: Combined effect of lirilumab and elotuzumab on KIR2D$^+$ NK cells towards multiple myeloma cell line U266B1 is seen *in vitro* on CD107 mobilization, and intracellular cytokines (TNF-α and IFN-γ) production

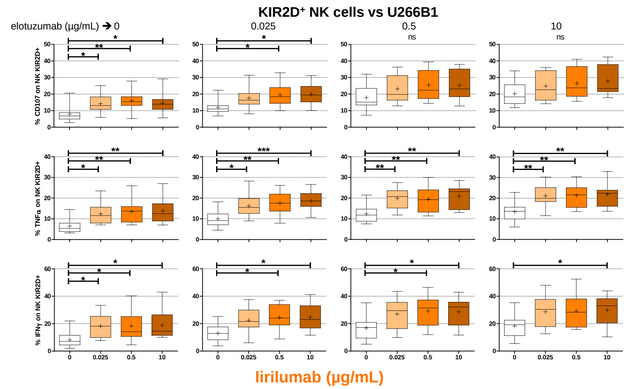


Fig. 3: CD107 mobilization (Top), intracellular TNF-α production (Middle) and intracellular IFN-γ production (bottom) in NK cells expressing KIR2D in response to U266B1 cells in 4h at 37° C, with indicated doses of lirilumab and elotuzumab. Box plots indicate the median with the second and third quartiles. Whiskers indicate min. and max. values. + indicates the mean. N=12 healthy volunteers.

Groups were compared using a one-way ANOVA and a Bonferroni multiple comparison test. ns = non significant, * p<0,05, ** p<0,01, *** p<0,001. Similar results were obtained with OPM-2 multiple myeloma cells. No significant effect of CD16 genotyping was observed in these experiments (data not shown).

Figure 4: Lirilumab enhances *in vivo* anti-tumor efficacy of elotuzumab

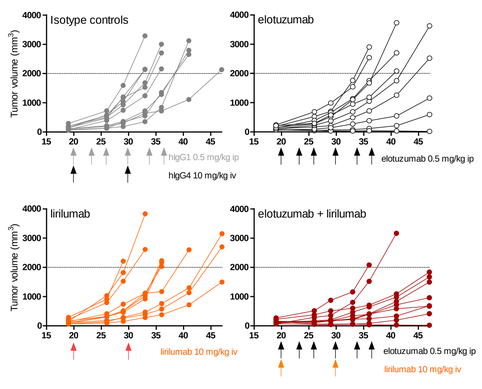


Fig. 4: KIR-cw3-Tg RAG mice were sc engrafted with 5x10^6 OPM-2 cells with matrigel. 19 days post tumor engraftment, mice were randomized for a mean tumor volume of 130 mm^3 (n=10 mice/group) and treated as indicated with arrows. Each curve corresponds to one individual tumor gowth. Data are representative of two independent experiments.

Figure 5: Combination therapy with lirilumab and elotuzumab significantly decreases tumor volumes

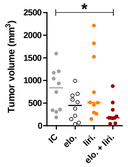


Fig. 5: Individual tumor volumes and medians on day 29 after tumor engraftment. Statistical comparision of medians of the tumor volumes performed with Kruskal-Wallis test followed with Dunn’s multiple comparision test, *, *P<0.05*.

References

Abstract #4717. *Effects of IL-21, KIR Blockade, and CD137 Agonism on the Non-Clinical Activity of Elotuzumab.*

Conclusions

We demonstrate that blockade of KIR checkpoint receptors with lirilumab was able to augment elotuzumab mediated ADCC *in vitro* and synergized with elotuzumab to mediate potent anti-MM activity *in vivo*.

These data suggest that lirilumab treatment may increase the therapeutic efficacy of elotuzumab:

- *In vitro* MM cells were capable of activating peripheral blood NK cells from healthy donors, responses were significantly enhanced, in a dose-dependent manner, by both lirilumab and elotuzumab independently
- The elotuzumab-mediated functional activation of KIR2D$^+$ NK cells could be further enhanced by the addition of increasing doses of lirilumab.
- *In vivo*, as monotherapy, each of monoclonal antibody had some therapeutic effect while the combination of both resulted in a significantly stronger anti-tumor effect and increased survival of the mice.

Taken together, these data provide a rationale for clinical trials to test combination treatment of lirilumab and elotuzumab in MM patients.

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Disclosures: Caroline Sola, Mathieu Bléry, Cécile Bonnafous,Elodie Bonnet, Nicolas Fuseri,Yannis Morel and Pascale André are employees of Innate Pharma. Robert F Graziano is employee of Bristol-Myers Squibb. All authors are equity owners in their respective companies.