Anti-tumoral efficacy of therapeutic human anti-KIR antibody (lirilumab) in a preclinical xenograft tumor model

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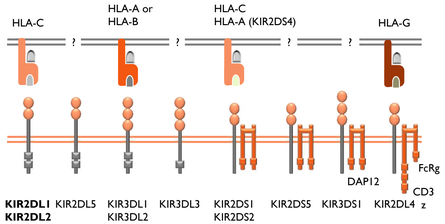
Introduction

- Natural Killer cells (NK cells) are lymphocytes able to recognize and kill tumors for which the expression of Major Histocompatibility Complex (MHC) class I molecules is altered.
- This "missing self" recognition is mediated by the lack of engagement of MHC class I molecules with NK inhibitory receptors that include lectin Ly49 molecules in mice and Killer Immunoglobulin like Receptors (or KIR) in humans. Blocking interactions between KIR and MHC class I positive tumor cells with monoclonal antibodies (mAb) constitutes an interesting therapeutic strategy.
- The anti-KIR2DL1/2/3-specific monoclonal antibody, lirilumab, is a human IgG4 that is being developed for treating both hematologic malignancies and solid tumors.
- **The objective of this study was to develop a preclinical model to assess the efficacy of the drug candidate tested in clinical trials, lirilumab.**

The KIR molecules and their ligands



(Adapted from Thielens *et al.*, Curr. Op. Immunol. 2012)

Results

HLA-C-expressing tumor escapes NK cell control in KIR tg-RAG mice

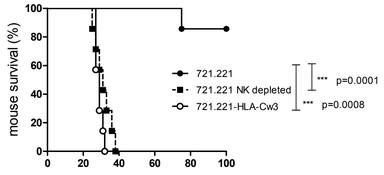


Fig 1: 721.221 or 721.221 HLA-Cw3 tumor cells (10^7 cells) were injected i.v. into KIR tg-RAG mice. When indicated, NK cell depletion was achieved by injecting i.v. 100 µg of anti-NK1.1 every 10 days, starting on the day of tumoral challenge.
Mouse survival was monitored for 100 days after tumoral challenge (results of a representative experiments are shown, n = 6 or 7mice per group). Differences in median survival were analyzed statistically with Log-Rank (Mantel Cox) test.

Duration of KIR receptor saturation is lirilumab dose-dependent in KIR tg-RAG mice

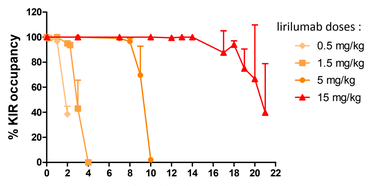
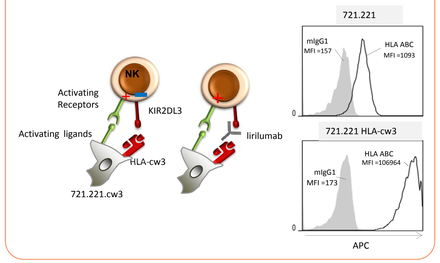


Fig 2: 0.5, 1.5, 5 or 15 mg/kg of lirilumab were injected i.v. to KIR tg-RAG mice (n=3 to 8). KIR receptor occupancy on NK was assessed by FACS on peripheral blood, using PE-conjugated lirilumab (Innate Pharma), anti-NK1.1-APC (PK136, BD Pharmingen).

Tools

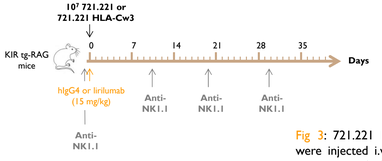
- tumor cells: the human B lymphoma cell line, 721.221, transduced or not with HLA-Cw3 molecule, a ligand of KIR2DL3.
- KIR tg-RAG mice expressing the human NK inhibitory KIR2DL3, on the surface of NK cells on a RAG-1 deficient background.
- lirilumab: anti-KIR2DL1/2/3 specific human IgG4 monoclonal antibody.



Conclusions

- Lirilumab treatment increased mice survival in a dose dependent manner when injected at the same time as the tumor challenge.
- This protective effect was NK cell mediated and directly correlated with the duration of KIR saturation.
- Interestingly, lirilumab treatment also improved survival in therapeutic conditions (i.e. when the antibody was injected 5 days after the tumor, also in a NK cell dependent manner).
- In conclusion, this study showed efficacy of lirilumab, even in therapeutic condition, as single agent in a HLA-Cw3-expressing tumor model and this xenogenic pre-clinical model will be an excellent tool to investigate the therapeutic benefits of combination treatments

Preventive lirilumab treatment improves mouse survival in a NK cell-dependent manner…



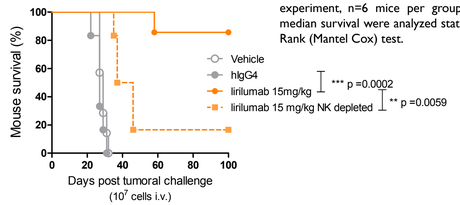
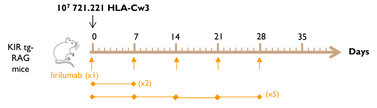


Fig 3: 721.221 HLA-Cw3 tumor cells (10^7 cells) were injected i.v. to KIR tg-RAG mice. Lirilumab was injected (i.v.) on day 0. When indicated, NK cell depletion was achieved by injecting i.v. 100 µg of anti-NK1.1 (PK136, mIgG2a) every 10 days, starting the day before tumoral challenge. (results of 1 experiment, n=6 mice per group). Differences in median survival were analyzed statistically with Log-Rank (Mantel Cox) test.

…and this effect is dependent on KIR saturation duration



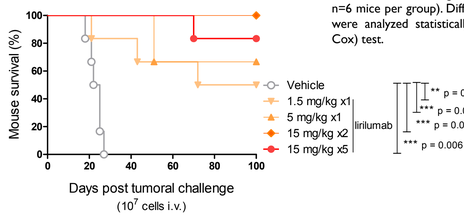
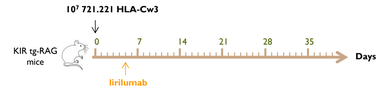


Fig 4: 721.221 HLA-Cw3 tumor cells (10^7 cells) were injected i.v. to KIR tg-RAG mice. Lirilumab injections (i.v.) were started on day 0.
Mouse survival has been monitored for 100 days after tumoral challenge (results of 1 experiment, n=6 mice per group). Differences in median survival were analyzed statistically with Log-Rank (Mantel Cox) test.

Therapeutic lirilumab treatment improves mouse survival



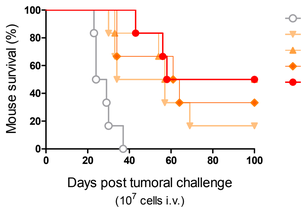
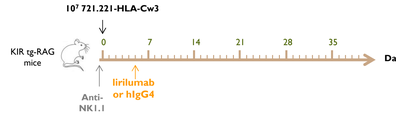


Fig 5: 721.221 HLA-Cw3 tumor cells (10^7 cells) were injected to KIR tg-RAG mice. Lirilumab was injected 5 days after tumor cells graft at the indicated doses.
Mouse survival has been monitored for 100 days after tumoral challenge (results of a representative experiment are shown, n=6 mice per group per exp).

		lirilumab (mg/kg, day 5)			
	Vehicle	0.5	1.5	5	15
Median Survival (days)	26.5	45.5	57	63	64.5
Increase Life Span (%)	-	71.7	115.1	130.2	143.4
Log-rank (Mantel-Cox) test	-	0.0168*	0.0016**	0.0097**	0.0005***

Lirilumab therapeutic efficacy is mediated by NK cells



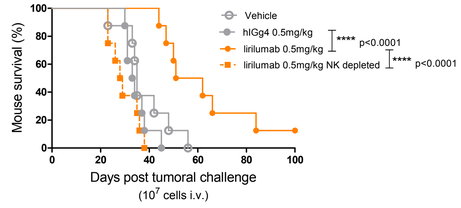


Fig 6: 721.221-HLA-Cw3 tumor cells (10^7 cells) were injected i.v. to KIR tg-RAG mice. 0.5 mg/kg of lirilumab (hIgG4) or an isotypic control mAb were injected i.v. on day 0. When indicated, NK cell depletion was achieved by injecting i.v. 100 µg of anti-NK1.1 (PK136, mIgG2a) the day before tumoral challenge (results of a representative experiment are shown, n=6 or 7 mice per group). Differences in median survival were analyzed statistically with Log-Rank (Mantel Cox) test.