Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies

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Abstract

- Natural killer (NK) cells mediate anti-lymphoma activity by spontaneous cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) when triggered by rituximab, an anti-CD20 monoclonal antibody used to treat patients with B cell lymphomas. The balance of inhibitory and activating signals determines the magnitude of NK cell’s efficacy by spontaneous cytotoxicity.

- Here, using a killer cell immunoglobulin-like receptor (KIR) transgenic murine model, we show that blockade of the interface of inhibitory KIRs with MHC class I antigens on lymphoma by anti-KIR antibodies prevents tolerogenic interaction and augments NK cell spontaneous cytotoxicity.

- In combination with anti-CD20 mAbs, anti-KIR treatment induces enhanced NK cell-mediated, rituximab-dependent cytotoxicity against lymphoma in vitro and in vivo in KIR transgenic and syngeneic murine lymphoma models.

- These results support a therapeutic strategy of combination, rituximab and KIR blockade through lirilumab, illustrating the potential efficacy of combining a tumor targeting therapy with an NK cell agonist thus stimulating the post-rituximab anti-lymphoma immune response.

Results

Anti-Ly49C/I F(ab')2 increases anti-CD20 mAb-mediated NK cell degranulation and cytotoxicity

- Purified NK cells from C57BL/6 mice were analyzed: (A) for degranulation by IFN-γ (pg/mL) using a 4 hour culture in the conditions described above (\(B, *p = 0.0109\)), and (C) in cytotoxicity assays using anti-Ly49C/I F(ab')2 (10 µg/mL), tumor, anti-CD20 mAb (CAT13, 10 µg/mL), or tumor, anti-CD20 mAb and anti-Ly49C/I F(ab')2 on day 3 and weekly for three weeks, or the combination of both (\(\*p = 0.0119\)).

- Lirilumab therapy improves survival in a therapeutic HLA+ tumor model in an NK cell-dependent manner

- Binyamin L. et al., 2008

- We present here pre-clinical evidence supporting another novel approach to enhancing mAb antitumor efficacy.

- Our findings provide rationale for the combination of an anti-KIR mAb to “remove the brakes” from NK cells and further enhance the efficacy of rituximab among other mAbs by renamement of ADCC.

- A clinical trial investigating this strategy is needed.

References

- Beldafox A. et al., Blood 2011; 118 (23): 6189-98
- Romagné F. et al., Blood 2011; 118 (23): 6182-9

Conclusions

- We present here pre-clinical evidence supporting another novel approach to enhancing mAb antitumor efficacy.

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Mechanism of action

Anti-KIR mAbs block an inhibitory signal to NK cells and enhance ADCC (antibody-dependent cell-mediated cytotoxicity) in vivo and in NK cell-dependent

- Anti-Ly49C/I F(ab')2 enhances the anti-lymphoma activity of anti-CD20 mAb in vivo and is NK cell-dependent

- Anti-Ly49C/I F(ab')2 increases anti-CD20 mAb-mediated NK cell degranulation and cytotoxicity

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- A-B) Post-tumor inoculation, mice received either flat control (\(\bullet\)), 250 µg of anti-CD20 mAb (CAT-13) on day 3 and weekly for three weeks (\(\triangle\)), 250 µg of anti-HLA-C mAb (G8-105), 250 µg of anti-HLA-DR mAb (7A8), 250 µg of anti-HLA-FcRIIIA mAb (9E10), or 250 µg of anti-CD20 mAb and 200 µg of anti-Ly49C/I F(ab')2 on day 3 (\(\triangle\)) and biweekly for three weeks, or the combination (\(\bullet\)). Mice were then monitored for overall survival (\(B, *p = 0.042\)).

- (C-D) Post-tumor inoculation, mice received either flat control (\(\bullet\)), 250 µg of anti-CD20 mAb (CAT-13) on day 3 and weekly for three weeks, or the combination (\(\bullet\)). Mice were then monitored for overall survival (\(B, *p = 0.042\)).

- B) Lirilumab therapy improves survival in a therapeutic HLA+ tumor model in an NK cell-dependent manner

- Rat IgG control on day 3 (\(\triangle\)), lirilumab at 0.5 mg/kg on day 5 and 100 µg anti-NK1.1 mAb on day 10, 15, 20, and 25 with 250 µg of anti-CD20 mAb and 200 µg anti-Ly49C/I F(ab')2 on day 3 (\(\triangle\)) with 250 µg of anti-NK1.1 mAb on day 5 (\(\triangle\)) to deplete NK cells in mice inoculated with EL4-huCD20

- C) Lirilumab therapy enhances the anti-lymphoma activity of rituximab in vivo and is NK cell-dependent

- A-B) Starting five days after tumor challenge, either no treatment (\(\bullet\)), isotype control at 0.5 mg/kg (\(\triangle\)), lirilumab at 0.5 mg/kg (\(\triangle\)), or lirilumab at 0.5 mg/kg and 100 µg anti-HA anti-NK1.1 mAb on day 10 with 250 µg of anti-CD20 mAb (CAT-13) on day 3 and weekly for three weeks, or the combination (\(\bullet\)). Mice were then monitored for overall survival (\(C, *p = 0.002\)).

- B) We present here pre-clinical evidence supporting another novel approach to enhancing mAb antitumor efficacy.

- Our findings provide rationale for the combination of an anti-KIR mAb to “remove the brakes” from NK cells and further enhance the efficacy of rituximab among other mAbs by renamement of ADCC.

- A clinical trial investigating this strategy is needed.

- C) Lirilumab therapy enhances the anti-lymphoma activity of rituximab in vivo and is NK cell-dependent

- ▲) Printed NK cells from C57BL/6 mice were analyzed: (A) for degranulation by IFN-γ (pg/mL) using a 4 hour culture in the conditions described above (\(B, *p = 0.0109\)), and (C) in cytotoxicity assays using anti-Ly49C/I F(ab')2 (10 µg/mL), tumor, anti-CD20 mAb (CAT13, 10 µg/mL), or tumor, anti-CD20 mAb and anti-Ly49C/I F(ab')2 (10 µg/mL) on day 3, or the combination of both (\(\*p = 0.0119\)).