A surrogate to the anti-IL1RAP antibody nadunolimab induces tumor microenvironment changes to the metastatic lung and reduces metastatic lesions in mouse models of metastatic cancer

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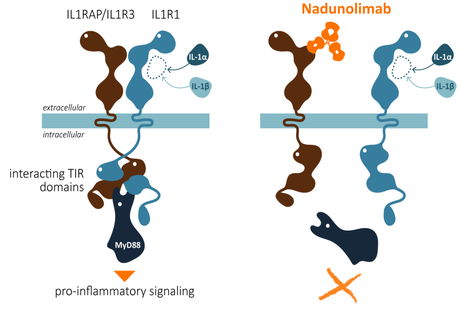
Introduction

Interleukin-1 Receptor Accessory Protein (IL1RAP) is a co-receptor for the IL1 receptor (IL1R1) and is required for IL1α and IL1β signaling. IL1RAP is expressed in the tumor microenvironment (TME); on cancer cells, stromal cells and on infiltrating immune cells in several types of cancers, including non-small cell lung cancer (NSCLC), pancreatic cancer (PDAC), triple-negative breast cancer (TNBC) and metastatic lesions in these indications.

Nadunolimab (CAN04) is a fully humanized ADCC-enhanced IgG1 antibody targeting IL1RAP and blocking both IL1α and IL1β signaling. Nadunolimab is currently evaluated in combination with chemotherapy in phase I/II clinical trials in NSCLC and PDAC (NCT03267316) and in TNBC (NCT05181462). Interim efficacy data for PDAC and NSCLC show increased OS and PFS compared to that expected from chemotherapy alone based on historical controls. In 73 PDAC patients (modified intention to treat, mITT analysis set) the median iPFS was 7.2 months and the median OS was 12.9 months, while in a total of 30 NSCLC patients (mITT analysis set) the overall response rate was 53%, with a median PFS of 6.8 months. Similarly, an initial analysis of the phase Ib part of the TNBC trial (n=12 patients) showed a favorable safety profile and a preliminary response rate of 50%, which compares favorably to the historical response rate of approximately 30% reported for chemotherapy alone[1].



Objectives

To assess the effects of the murine surrogate antibody to nadunolimab on the metastatic TME using the metastatic 4T1 murine TNBC and B16-F10-luc i.v. models.

Results

Treatment with the nadunolimab surrogate antibody reduces the metastatic burden in the murine 4T1 and B16-F10-luc i.v. models

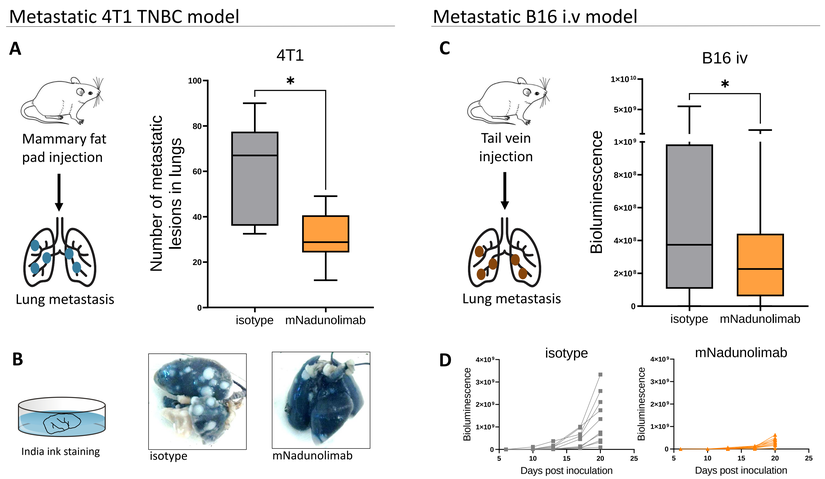


Figure 1. To assess the effect of the nadunolimab surrogate antibody (mNadunolimab) on metastasis, we used the murine lung metastatic TNBC 4T1 and B16-F10-luc i.v. models. Balb/c mice were inoculated in the mammary fat pad with 10^5 4T1 cells and the number of metastatic lesions were assessed at day 28 in isotype (n=7) or mNadunolimab treated (n=8) mice (10 mg/kg, BID on day 7 post cell inoculation, then BIW). Boxplot (A) and india ink coloration photographs (B) show reduced metastatic burden in mice treated with mNadunolimab. One representative experiment out of 5 experiments depicted. C57BL/6J albino were injected i.v. with $5x10^5$ B16-F10-luc cells and the metastatic burden was measured with bioluminescence every 3-4 days. Boxplot of bioluminescence measurements at endpoint (19-20 days) from 4 experiments combined (C) and bioluminescence curves from one representative experiment (D) show that mNadunolimab reduced the metastatic burden also in the B16-F10 model.

TME infiltrating myeloid cells have upregulated IL1RAP expression

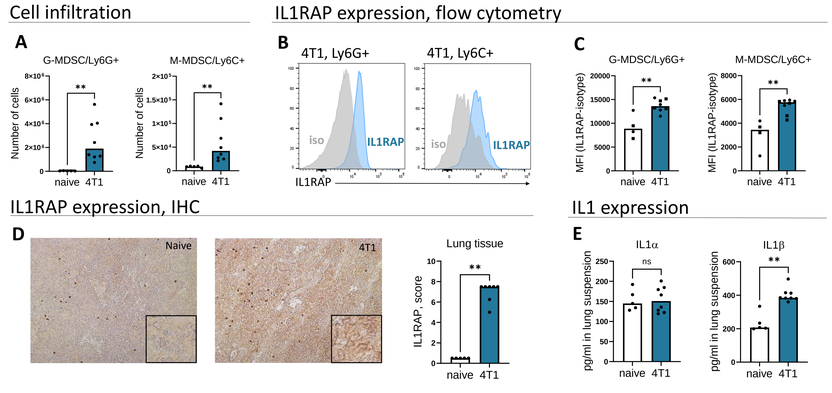


Figure 2. To gain insight into IL1RAP and IL1 expression in metastatic lungs in the 4T1 TNBC model, Balb/c mice were inoculated with 4T1 cells as previously described. On day 18, lung tissue was collected, and single cell suspension prepared for flow cytometry analysis of infiltrating immune cells and their IL1RAP expression. A large influx of myeloid cells was observed (A). Notably, infiltrating Ly6G+ and Ly6C+ cells had a distinct upregulation of IL1RAP compared to Ly6G+ and Ly6C+ cells from naïve lungs (B-C). In parallel, lung tissue was also collected for IHC, which confirmed the elevated levels of IL1RAP (D). Cell pellet supernatants obtained from lung tissue single-cell suspensions were analyzed by Luminex for IL1α and IL1β levels. Both IL1α and IL1β were present in the metastatic lungs, with levels of IL1β being increased in comparison to naïve mice (E).

IL1 induces secretion of a large set of cytokine and chemokines from different cell types which can be blocked by the nadunolimab surrogate antibody

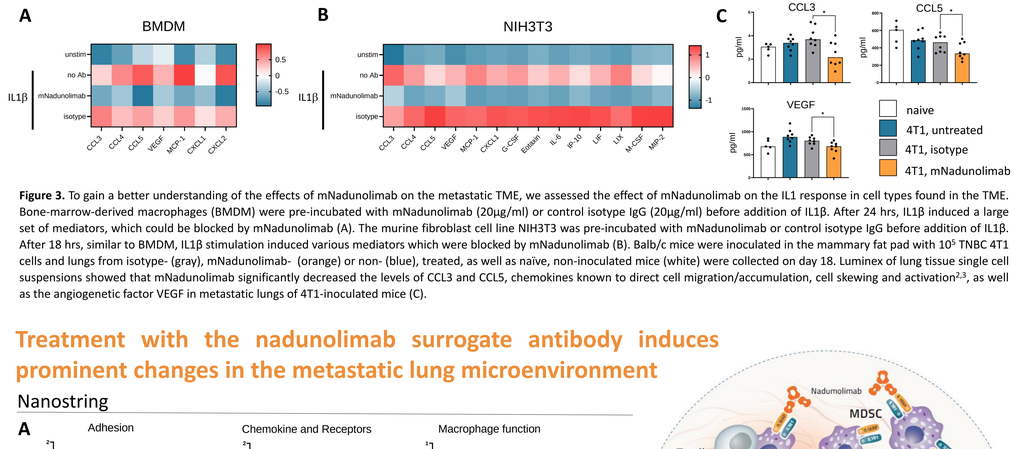


Figure 3. To gain a better understanding of the effects of mNadunolimab on the metastatic TME, we assessed the effect of mNadunolimab on the IL1 response in cell types found in the TME. Bone-marrow-derived macrophages (BMDM) were pre-incubated with mNadunolimab (20μg/ml) or control isotype IgG (20μg/ml) before addition of IL1β. After 24 hrs, IL1β induced a large set of mediators, which could be blocked by mNadunolimab (A). The murine fibroblast cell line NIH3T3 was pre-incubated with mNadunolimab or control isotype IgG before addition of IL1β. After 18 hrs, similar to BMDM, IL1β stimulation induced various mediators which were blocked by mNadunolimab (B). Balb/c mice were inoculated in the mammary fat pad with 10^5 TNBC 4T1 cells and lungs from isotype- (gray), mNadunolimab- (orange) or non- (blue), treated, as well as naïve, non-inoculated mice (white) were collected on day 18. Luminex of lung tissue single cell suspensions showed that mNadunolimab significantly decreased the levels of CCL3 and CCL5, chemokines known to direct cell migration/accumulation, cell skewing and activation[2,3], as well as the angiogenetic factor VEGF in metastatic lungs of 4T1-inoculated mice (C).

Treatment with the nadunolimab surrogate antibody induces prominent changes in the metastatic lung microenvironment

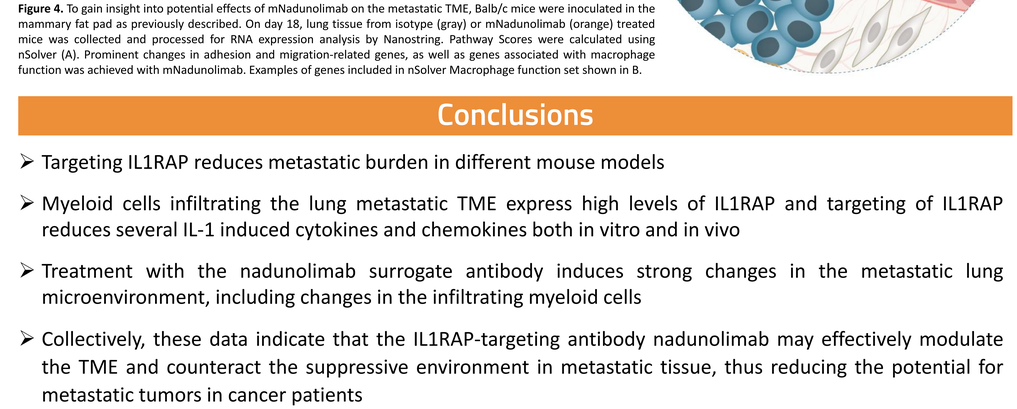


Figure 4. To gain insight into potential effects of mNadunolimab on the metastatic TME, Balb/c mice were inoculated in the mammary fat pad as previously described. On day 18, lung tissue from isotype (gray) or mNadunolimab (orange) treated mice was collected and processed for RNA expression analysis by Nanostring. Pathway Scores were calculated using nSolver (A). Prominent changes in adhesion and migration-related genes, as well as genes associated with macrophage function was achieved with mNadunolimab. Examples of genes included in nSolver Macrophage function set shown in B.

Conclusions

- Targeting IL1RAP reduces metastatic burden in different mouse models
- Myeloid cells infiltrating the lung metastatic TME express high levels of IL1RAP and targeting of IL1RAP reduces several IL-1 induced cytokines and chemokines both in vitro and in vivo
- Treatment with the nadunolimab surrogate antibody induces strong changes in the metastatic lung microenvironment, including changes in the infiltrating myeloid cells
- Collectively, these data indicate that the IL1RAP-targeting antibody nadunolimab may effectively modulate the TME and counteract the suppressive environment in metastatic tissue, thus reducing the potential for metastatic tumors in cancer patients

References

[1] O'Shaughnessy, J Clin Oncol (2014), [2] Argyle, Kitamura, Front Immunol (2018), [3] Luther, Cyster, Nature Immunol (2001)