NT219 induces tumor PD-L1 expression and potentiates anti-PD-1 efficacy

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Background

While the advent of immune checkpoint blockade (ICB) has dramatically improved the prognosis of many immune-infiltrated cancers, for others, unfortunately, these benefits have yet to be realized. The major challenge before the field, then, is to identify combination therapies that act both to combat evolved resistance. NT219 is a novel dual inhibitor of insulin receptor substrates 1 and 2 (IRS) and STAT3. NT219 demonstrated antitumor effects against both in situ and metastatic human melanoma models in mice as a stand-alone treatment and in combination with mutated BRAF and MEK inhibitors. The potential of NT219 to overcome resistance and increase efficacy was demonstrated in PDX models with multiple drug classes. Collectively, these findings provided preclinical proof-of-concept NT219 as a promising novel cancer therapy. Given this data, the goal of our study was to assess the efficacy of combining NT219 with anti-PD-1 and anti-CTLA-4 ICB, and test capacity of the combination to overcome immune resistance

Methods

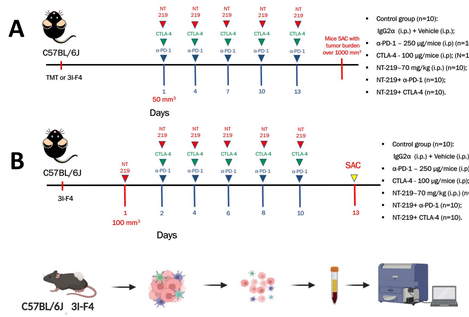


Figure 1: In vivo experimental design and treatment schedule.
(A) Immunocompetent C57BL6 mice were subcutaneously engrafted with B16-tdTomato (TMT) and B16 3I-F4 tumors, ICB-sensitive and ICB-resistant clones of mouse melanoma B16, respectively. Five days later treatments were initiated with the indicated treatments and mice were monitored for tumor growth (10 mice per group, n=10). (B) Immunocompetent C57BL6 mice were subcutaneously engrafted with ICB-resistant B16 3I-F4 tumors, and 13 days later treatments initiated with either control, NT219, α-PD-1, anti-CTLA4 antibodies, or the combination of NT219 with α-PD-1 or NT219 with anti-CTLA4. Three days following 5 cycles of treatment (Days 13), mice were sacrificed, and immune profiling of the tumors was performed by high content flow cytometry on BD X-30 to quantify tumor infiltrating myeloid and T cell repertoires.

NT219 promotes PD-L1 expression in ICB-resistant 3I-F4 cells

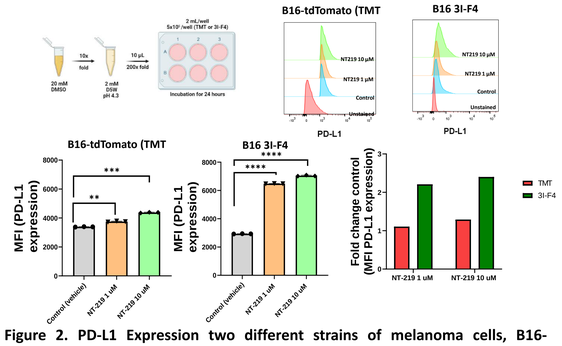


Figure 2. PD-L1 Expression two different strains of melanoma cells, B16-tdTomato (TMT) and B16 3I-F4, ICB sensitive and ICB resistant derivative lines, respectively. NT219 induced significantly higher PD-L1 expression in resistant melanoma cells compared to B16-tdTomato ICB-sensitive cells, suggesting its potential to re-sensitizes ICB refractory tumor.

ICB-resistant 3I-F4 cells express higher levels of IRS1 and pSTAT3 than the ICB-sensitive cells, both abolished by NT219

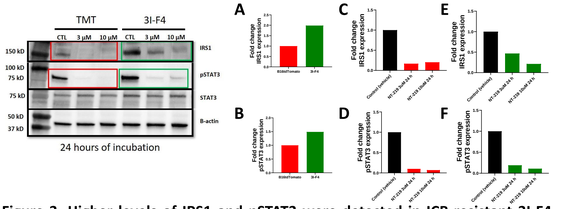


Figure 3. Higher levels of IRS1 and pSTAT3 were detected in ICB-resistant 3I-F4 B16 strain as compared to ICB-sensitive B16-tdTomato (TMT) strain (A-B).
The suppression of both IRS1 and pSTAT3 by the dual inhibitor NT219 is demonstrated in both cell lines (C-F).

NT219 synergizes with anti-PD-1 to inhibit ICB-sensitive tumor growth

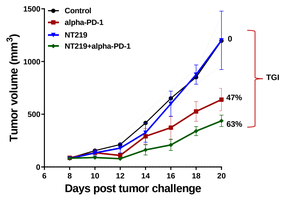


Figure 4. NT219 and anti-PD-1 combination synergize to induce significant tumor growth inhibition (63%, p value 0.01) of ICB-sensitive B16 tdTomato (TMT) subcutaneous tumors. Each curve represent the mean value of 10 mice per treatment group.

NT219 sensitizes resistant tumors to αPD-1 and prolongs mouse survival in ICB-resistant model

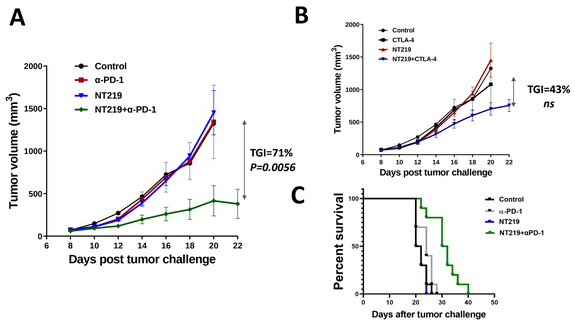


Figure 5. (A-B) Tumor growth inhibition in B16 3I-F4 ICB resistant model. The indicated treatments were administered twice a week. Each curve represents the mean tumor volume in 10 mice per treatment group (n=10). (C) Survival curves of C57BL6 mice bearing 3I-4F tumors, treated until day 13; tumor volume > 1000mm³ was the endpoint.

The combination of NT219 and Pembrolizumab re-sensitizes α-PD1 refractory tumors in humanized PDX of gastroesophageal cancer

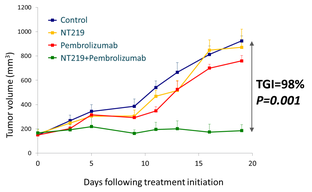
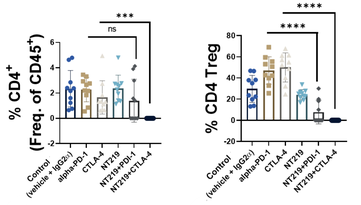


Figure 6. Combined treatment of NT219 with α-PD1 inhibited the growth of α-PD1 refractory tumors (TGI = 98%, p= 0.001) in a humanized PDX model of GEJ cancer. Both the tumor and the PBMCs were derived from the same patient (autologous model). PBMCs were injected to the mice and treatments initiated on Day 0. The mice (3 per group) were treated on days 0,5 and 10.

NT219 cooperates with α-PD-1 or α-CTLA4 to suppress Treg frequency in the TME

Figure 7. NT219 in combination with either α-PD-1 or α-CTLA4 significantly decreases CD4 Treg infiltration in ICB resistant B16 3I-F4 tumor microenvironment, while none of the monotherapies showed such an effect.



Combination therapy improves CD8⁺ Teff cell infiltration and decreases the PD-1⁺CD8 T cell fraction in the TME

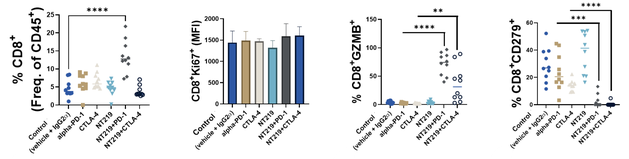


Figure 8. The combined therapy of NT219 with α-PD-1 or α-CTLA4 enhances the antitumor immune response in ICB-resistant model by reprogramming the tumor microenvironment to support a higher proportion of activated CD8 T cells, while decreasing the proportion of PD-1⁺CD8 T cell population. Statistical significance between groups were determined by ANOVA.

NT219 combined with α-PD1 or α-CTLA4 significantly enhances GzmB+ NK cell infiltration in ICB-resistant cancer

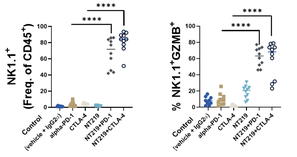


Figure 9. The combination of NT219 with either α-PD-1 or α-CTLA4 showed significant benefit in NK cell frequency and cytotoxic potential within the tumor microenvironment in ICB resistance model, while none of the treatments alone had any effect.

NT219 combined with α-PD1 or α-CTLA4 significantly decreases the T-cell suppressive myeloid subsets in the TME

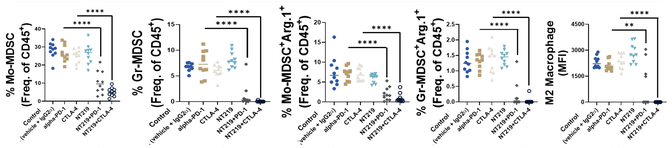


Figure 10. The combination of NT219 with either α-PD-1 or α-CTLA4 resulted in a significant decrease in monocytic and granulocytic MDSC infiltration, Arginase I expression, and M2 macrophage frequency in the tumor microenvironment, while none of the treatments alone had any effect.

Conclusion

Our findings provide a rationale for combining anti-PD-1 therapy with NT219 as a potential strategy to overcome resistance to immune checkpoint blockade (ICB) therapy.

Immune profiling of the ICB-resistant melanoma tumors following treatment of the mice with the NT219 combination with α-PD-1 or α-CTLA4 demonstrates reprograming of the TME and reveals enhanced infiltration and activation of cytotoxic T cells and NK cells, paralleled with a decrease in T regulatory cells, M2 macrophages and MDSCs, suggesting the potential of this approach to restore the efficacy of anti-PD-1 and anti-CTLA4 therapies and expand the patient population that can benefit from these drugs.

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