N17350 is an emerging therapeutic modality that selectively kills cancer cells and stimulates anti-tumor immunity

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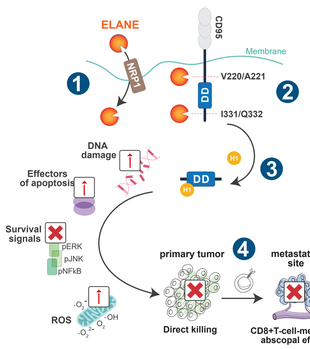
Introduction

Cancer is a disease driven by variable genetic mutations. Overcoming this varability while sparing normal cells has stymied broad-acting therapy development. Our innate immune system evolved to clear genetically diverse pathogens and limit host toxicity, raising the possibility that it can produce similar effects in cancer. Previous studies showed that neutrophil elastase (ELANE) – a neutrophil-derived serine protease – killed a wide range of cancer cells without harming non-cancer cells by cleaving CD95, the FAS receptor (Cui et al., Cell, 2021). ELANE attenuated primary tumor growth and produced a CD8+T cell-mediated abscopal effect to attack metastases. Here we leveraged this ELANE-mediated pathway to produce an optimized N17350 biologic and tested its effects on tumor development, both as a monotherapy and in combination with checkpoint inhibitors. Our findings underscore the viability of N17350 as a new therapeutic modality leveraging innate immunity.

ELANE-mediated anti-cancer pathway



1. **Cell uptake:** ELANE enters through the cell surface receptor Neuropilin 1 (NRP1)
2. **Broad Cancer Cell Killing:** ELANE proteolytically liberates the CD95 death domain (DD) to set-up unique & complex killing program
3. **Selective Cancer Cell Killing:** Interaction betweens histone 1 (H1) and the liberated DD mediate selective cancer cell killing
4. **Abscopal Effect:** ELANE attenuates tumor growth at metastatic sites via CD8+ T cell-mediated abscopal effect

Figure 1: Proposed Mechanism of Action of ELANE and N17350, adapted from Cui et al, Cell, 2021

Highlights on N17350

- selectively kills a wide range of cancer cells without harming non-cancer cells
- induces immunogenic cell death
- is unable to induce resistance following repeated treatment
- generates abscopal effects and immune memory in pre-clincial models
- synergizes with and/or enables an anti-CTLA4 antibody in hot and cold tumors

N17350 selectively kills a wide range of cancer cells, induces immunogenic cell death, and is unable to induce resistance following repeated treatment

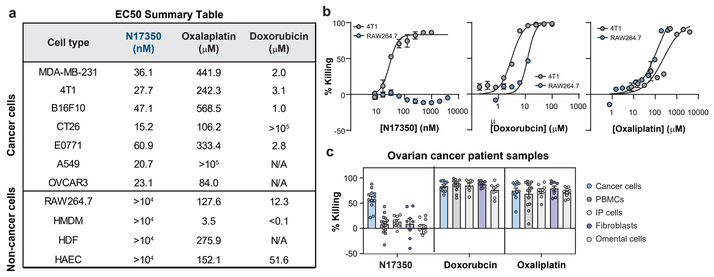


Figure 2. N17350 selectively kills cancer cells. Murine or human cancer and non-cancer cells were treated with N17350 for 24h, or doxorubicin or oxaliplatin for 72h. Cell killing was quantified by calcein-AM. **Panel a**: Summary of EC50 values. **Panel b**: Representative data for 4T1 and RAW264.7 cells. **Panel c**: Effects of N17350 (4μM), doxorubicin (100μM), or oxaliplatin (1mM) on cancer and non-cancer cells isolated from ovarian cancer patients.

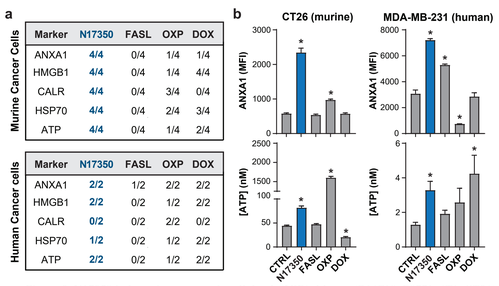


Figure 3. N17350 induces immunogenic cell death (ICD). Murine (B16F10, E0771, 4T1, CT26) or human (MDA-MB-231, A549) cancer cells were treated with N17350, FASL, oxaliplatin (OXP) or doxorubcin (DOX). **Panel a**: Summary of ICD markers in cancer cells (elevated/tested). **Panel b**: Representative data for ATP and ANXA1.

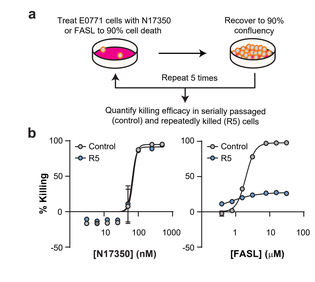


Figure 4. Cancer cells are not able to generate resistance to N17350 following repeated treament. Panel a: Experimental design. **Panel b**: N17350 and FASL killing efficacy of control and R5 cells.

N17350 induces durable tumor regression with favorable innate and adaptive immune profiles, abscopal effects, and immune memory in pre-clinical tumor models

CT26 "immunologically hot" model of colon cancer

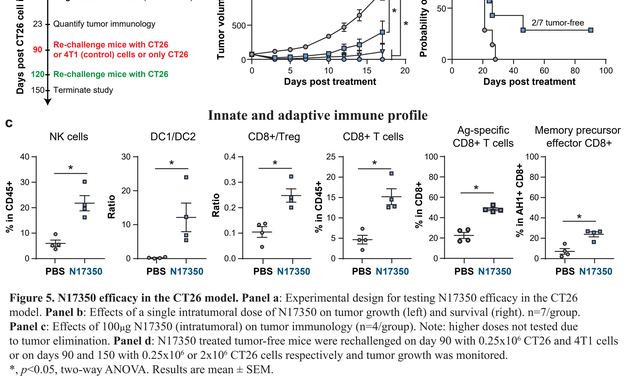


Figure 5. N17350 efficacy in the CT26 model. Panel a: Experimental design for testing N17350 efficacy in the CT26 model. **Panel b**: Effects of a single intratumoral dose of N17350 on tumor growth (left) and survival (right). n=7/group. **Panel c**: Effects of 100μg N17350 (intratumoral) on tumor immunology (n=4/group). Note: higher doses not tested due to tumor elimination. **Panel d**: N17350 treated tumor-free mice were rechallenged on day 90 with 0.25x10^6 CT26 and 4T1 cells or on days 90 and 150 with 0.25x10^6 or 2x10^6 CT26 cells respectively and tumor growth was monitored. *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

4T1 "immunologically cold" model of metastatic breast cancer

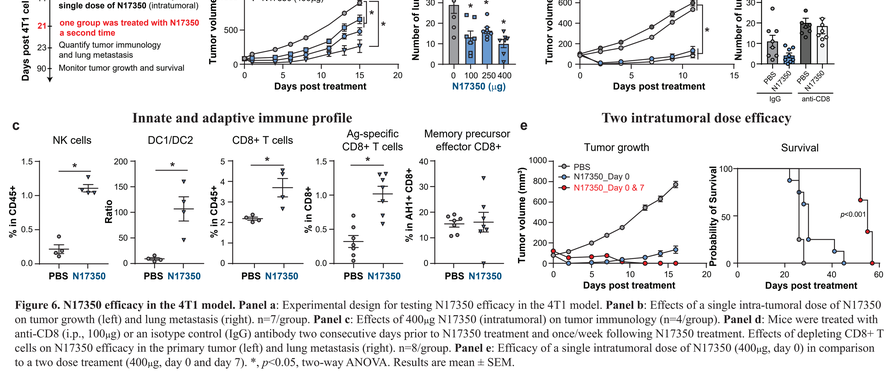


Figure 6. N17350 efficacy in the 4T1 model. Panel a: Experimental design for testing N17350 efficacy in the 4T1 model. **Panel b**: Effects of a single intra-tumoral dose of N17350 on tumor growth (left) and lung metastasis (right). n=7/group. **Panel c**: Effects of 400μg N17350 (intratumoral) on tumor immunology (n=4/group). **Panel d**: Mice were treated with anti-CD8 (i.p., 100μg) or an isotype control (IgG) antibody two consecutive days prior to N17350 treatment and once/week following N17350 treatment. Effects of depleting CD8+ T cells on N17350 efficacy in the primary tumor (left) and lung metastasis (right). n=8/group. **Panel e**: Efficacy of a single intratumoral dose of N17350 (400μg, day 0) in comparison to a two dose treament (400μg, day 0 and day 7). *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

N17350 synergizes with anti-CTLA4 in hot and cold tumors

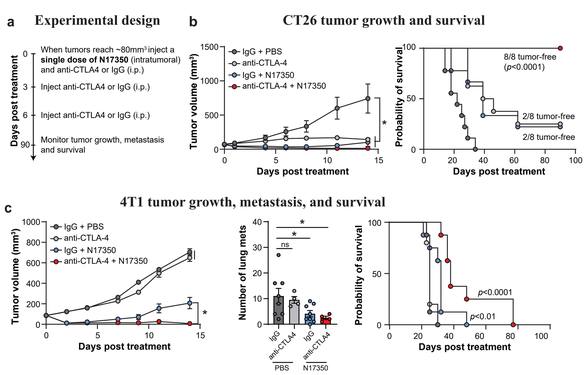


Figure 8. N17350 synergizes with anti-CTLA4 in the CT26 and 4T1 models.
Panel a: Experimental design for testing N17350 and anti-CTLA4 combination therapy. Tumors (~80mm³) were injected with N17350 on day 0; CT26: 100μg dose, 4T1: 400μg dose. Anti-CTLA4 or IgG isotype was injected intra-peritoneally (i.p.) on days 0, 3, and 6 (100μg/day). **Panel b**: Effects of N17350 and anti-CTLA4, alone or in combination, in the CT26 model (n=8/group). Left: tumor growth. Right: survival. **Panel c**: Effects of N17350 and anti-CTLA4, alone or in combination, in the 4T1 model (n=8/group). Left: tumor growth. Middle: lung metastasis. Right: survival. *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

N17350 shows improved efficacy compared to chemotherapies

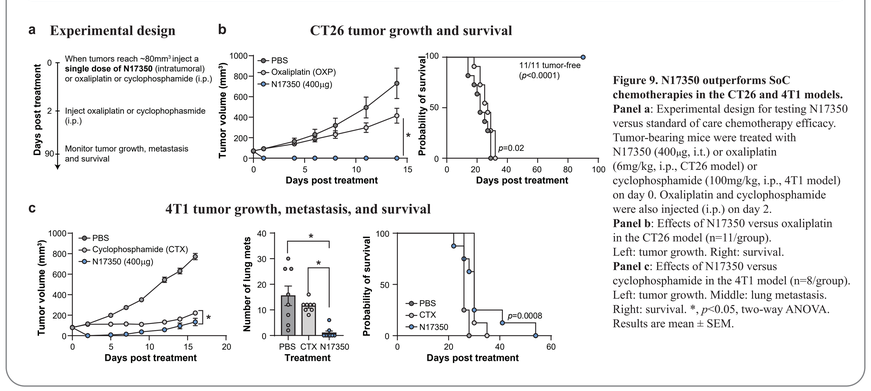


Figure 9. N17350 outperforms SoC chemotherapies in the CT26 and 4T1 models.
Panel a: Experimental design for testing N17350 versus standard of care chemotherapy efficacy. Tumor-bearing mice were treated with N17350 (400μg, i.t.) or oxaliplatin (6mg/kg, i.p., CT26 model) or cyclophosphamide (100mg/kg, i.p., 4T1 model) on day 0. Oxaliplatin and cyclophosphamide were also injected (i.p.) on day 2. **Panel b**: Effects of N17350 versus oxaliplatin in the CT26 model (n=11/group). Left: tumor growth. Right: survival. **Panel c**: Effects of N17350 versus cyclophosphamide in the 4T1 model (n=8/group). Left: tumor growth. Middle: lung metastasis. Right: survival. *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.