N17350 Combines Selective Cancer Killing with Adaptive Immune Activation to Eradicate Tumors

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BACKGROUND

Studies have shown that ELANE – a neutrophil-derived serine protease – kills a wide range of cancer cells without harming non-cancer cells by cleaving CD95 to liberate the death domain. This liberated death domain interacts with histone H1, whose elevated levels in cancer versus non-cancer cells contribute to selective cancer killing (Cui et al., Cell, 2021). Here we leveraged this ELANE-mediated pathway to develop an optimized N17350 biologic, tested its effects on tumor development as a monotherapy and in combination with checkpoint inhibitors, and evaluated histone H1 as a potential biomarker for N17350 efficacy. Our findings underscore the viability of N17350 as a new therapeutic modality.

MECHANISM OF ACTION
Histone H1-death domain axis

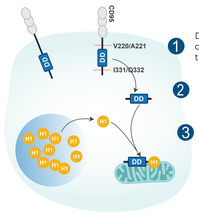


Figure 1: Proposed Mechanism of Action of ELANE and N17350, adapted from Cui et al, Cell, 2021

HIGHLIGHTS ON N17350

- Potent and direct cancer cell killing
- Selectivity over non-cancer cells
- Induces immunogenic cell death
- No induction of resistance following repeated treatment
- Generates abscopal effects and immune memory
- Synergizes with checkpoint inhibitors

Histone H1-death domain axis allows N17350 to selectively kill cancer cells independent of genotype and anatomical origin

N17350 selectively kills cancer cells which possess high histone H1 levels

Histone H1 levels correlate with N17350 killing capability

N17350 triggers histone H1 translocation in cancer cells

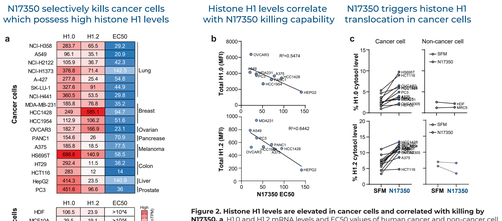


Figure 2. Histone H1 levels are elevated in cancer cells and correlated with killing by N17350. a. H1.0 and H1.2 mRNA levels and EC50 values of human cancer and non-cancer cell lines. RNA data was obtained from TCGA database. * TCGA data was unavailable; values were measured by qRT-PCR, normalized to A549 cells, and converted to nTPM. **b.** Correlation of H1.0 and H1.2 protein levels (quantified by flow cytometry) and N17350 EC50 values across multiple cancer cell lines. **c.** Cytosolic and total H1.0 and H1.2 levels in cancer cells pre- and post-treatment with serum-free media (SFM, control) or N17350 (200nM, 4h) were measured by flow cytometry. Results are expressed as percent cytosolic.

Experimental design

N17350 kills primary OvCa cells which posses high histone H1 Levels

N17350 effectively kills primary OvCa cells and spares non-cancer cells

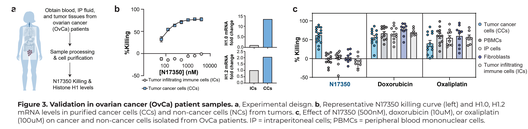


Figure 3. Validation in ovarian cancer (OvCa) patient samples. a. Experimental design. **b.** Representative N17350 killing curve (left) and H1.0, H1.2 mRNA levels in purified cancer cells (CCs) and non-cancer cells (NCs) from tumors. **c.** Effect of N17350 (500nM), doxorubicin (10uM), or oxaliplatin (100uM) on cancer and non-cancer cells isolated from OvCa patients. IP = intraperitoneal cells; PBMCs = peripheral blood mononuclear cells.

N17350 effectively regresses tumors in multiple pre-clinical models

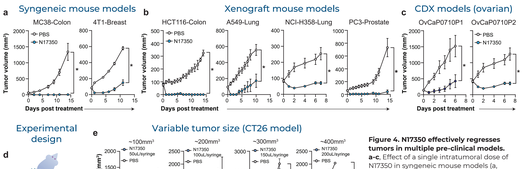


Figure 4. N17350 effectively regresses tumors in multiple pre-clinical models. a–c. Effect of a single intratumoral dose of N17350 in syngeneic mouse models (a, n=7/group), xenograft mouse models (b, n= 5/group), and ovarian patient cell-derived xenograft models (c, n=5/group). **d–e.** Experimental design (d) and effects of a single intratumoral dose of N17350 on larger CT26 tumors (e, n=5/group). *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

N17350 induces immunogenic cell death in cancer cells and mobilizes anti-tumor immunity

Summary of immune activating effects of N17350

N17350 induces immunogenic cell death in human and murine cancer cells

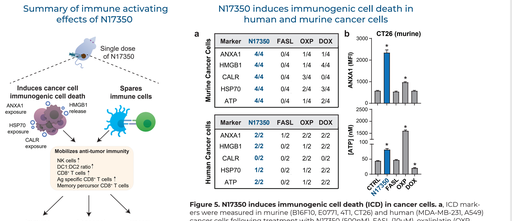


Figure 5. N17350 induces immunogenic cell death (ICD) in cancer cells. a. ICD markers were measured in murine (B16F10, E0771, 4T1, CT26) and human (MDA-MB-231, A549) cancer cells following treatment with N17350 (500nM), FASL (10uM), oxaliplatin (OXP, 100uM) or doxorubicin (DOX, 10uM). Results are number of cell types in which N17350 elevated ICD markers versus number of cell lines tested. **b.** Representative data for ATP and ANXA1 in CT26 cells. *, $p<0.05$, Student's t-test. Results are mean ± SEM.

N17350 induces favorable innate and adaptive immunology in hot and cold tumors

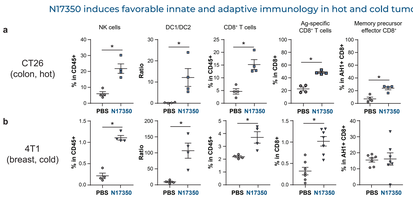
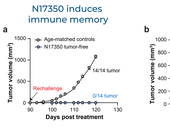


Figure 6. N17350 induces favorable innate and adaptive immune response. a. Effects of 100ug N17350 on tumor immunology 12 days post-treatment in the CT26 model (n=4/group). **b.** Effects of 400ug N17350 on tumor immunology 12 days post-treatment in the 4T1 model (n=4/group). *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

N17350 induces immune memory



N17350 induces a CD8⁺ T cell-mediated abscopal effect

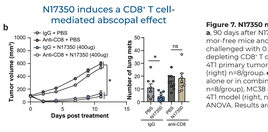
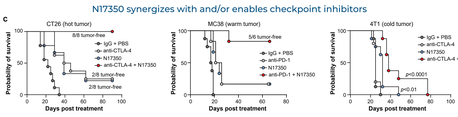


Figure 7. N17350 mobilizes anti-tumor immunity. At 90 days after N17350 treatment, CT26 tumor-free mice and age matched controls were re-challenged with 0.25x10⁶ CT26 cells. **b.** Effects of depleting CD8⁺ T cells on N17350 efficacy in the 4T1 primary tumor (left) and lung metastases (right) n=8/group. **c.** Effects of N17350 and CPI, alone or in combination, in the CT26 model (left, n=8/group), MC38 model (middle, n=6/group), and 4T1 model (right, n=8/group). *, $p<0.05$, two-way ANOVA. Results are mean ± SEM.

N17350 synergizes with and/or enables checkpoint inhibitors



N17350 is unable to induce resistance following repeated dosing in vitro and in vivo

Repeated treatment in vitro

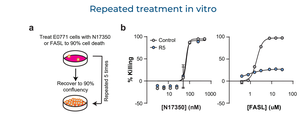


Figure 8. Cancer cells are not able to generate resistance to N17350 following repeated treament. a. Experimental design. **b.** N17350 and FASL killing efficacy of control and cancer cells exposed to 5 rounds of killing (RS).

Repeated dosing in vivo

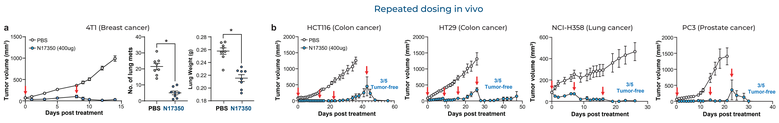


Figure 9. Repeated intratumoral dosing with N17350 eliminates tumors in multiple pre-clinical models. a. Tumor growth in the 4T1 model (injected on Days 0, 8). Tumor growth (left) and lung metastases (right). **b.** Tumor growth in the HCT116 model (injected on Days 0, 10, 22, 44); HT29 model (injected on Days 0, 8, 16, 26), NCI-H358 model (injected on Days 0, 8, 16), and PC3 model (injected on Days 0, 14, 23). Red arrow indicates treatement days. n=5/group. *, $p<0.05$, Student's t-test. Results are mean ± SEM.

METHODS

For in vitro studies, human and murine cancer and non-cancer cells (cell lines, primary cells from healthy donors and ovarian cancer patients) were treated with N17350 and cell viability was quantified by calcein-AM and immunogenic cell death (ICD) markers. To evaluate histone H1 as a potential biomarker, we studied the correlation between H1 levels in cancer cells and N17350 killing and compared H1 levels in normal and tumor tissues in patients. **For in vivo studies**, a single dose of N17350 was delivered intratumorally into multiple tumor models (syngeneic, xenograft, and CDX). Effects on primary and metastatic (4T1) tumor growth, immunology, and survival were assessed as a monotherapy or in combination with checkpoint inhibitors (anti-CTLA4 and anti-PD-1). CT26 tumor-free mice were rechallenged to examine immune memory.

RESULTS & CONCLUSION

Potent and direct cancer cell killing by N17350. N17350 selectively killed primary cancer cells from ovarian cancer (OvCa) patients and over 50 different cancer cell lines. The efficacy of N17350 killing was correlated with histone H1 isoform levels, which were upregulated in cancer cells versus non-cancer cells. A single intratumoral dose of N17350 regressed tumors in syngeneic, xenograft, and OvCa CDX models, spanning multiple cancer cell genetics, anatomical origin, and size. **Immune activation by N17350.** N17350 induced ICD markers in human and murine cancer cells in vitro, and increased favorable innate and adaptive immune cells in both hot and cold tumors in vivo. Functionally, this N17350-mediated immune response conferred protection from rechallenge in the immunologically hot CT26 model, produced a CD8⁺ T cell-mediated absocpal effect in the immunologically cold 4T1 model, and synergized with and/or enabled checkpoint inhibitor efficacy. **N17350 is unable to induce resistance**. Repeatedly exposing cancer cells to N17350 in vitro and in vivo does not lead to reduced killing capability.

Taken together, our data demonstrate that N17350 **selectively** kills cancer cells, produces **complete responses** in mice, induces favorable innate and adaptive immunology, and identify **histone H1** as an indicator of efficacy. Thus, further studies in a clinical setting are warranted.