Therapeutic potential of the TRIM21-Notch1 axis blockade in triple-negative breast cancer

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Introduction

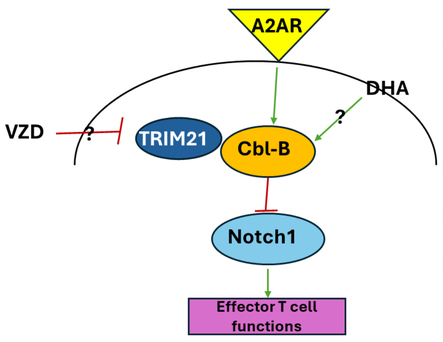

Figure 1: Trim21-Notch1 Axis regulation in T-cells. A2AR activation promotes Cbl-b-mediated Notch1 degradation. Pharmacological inhibition of Cbl-b restores Notch1 and effector T-cell functions, whereas Cbl-b activation or TRIM21 inhibition by DHA and VZD suppresses Notch1 and T-cell functions

Triple-negative breast cancer (TNBC) is an aggressive subtype comprising about 15% of breast cancer cases. Characterized by the absence of ER, PR, and HER2 receptors, TNBC lacks defined therapeutic targets, limiting treatment options and contributing to poor prognosis. Like many cancers, TNBC can evade immune detection: immunosuppressive signals in the tumor microenvironment can activate the adenosine A2A receptor (A2AR), leading to Cbl-b–mediated degradation of Notch1 in T-cells, leading to impaired immune function.

TRIM21, another E3 ligase, may regulate this pathway by modulating the activity of Cbl-b and in turn Notch1 signaling. In this study, we examined the effects of different drugs that may target this regulatory axis: we tested the effects of Dihydroartemisinin (DHA), Vilazodone (VZD), CGS-21680, and NTX-801 on T-cell function, Notch1 expression, and tumor progression in TNBC, with a focus on the TRIM21-Notch1 axis as a potential therapeutic target.

Methods

- **Cell Culture and Treatments**
  - Primary T-cells were isolated from the spleen of immunocompetent mice (FVB) using a negative selection kit. T-cells were activated with anti-CD3 and anti-CD28 antibodies for 48h.
  - Cells were treated with:
    - Dihydroartemisinin (DHA), (anti-malarial drug known for its anti-inflammatory and anticancer potential) ; 1µM
    - Vilazodone (VZD) (antidepressant with partial serotonin receptor activity being explored for off-target immunomodulatory effects); 10 µM
    - CGS-21680 (A2AR agonist); 1µM
    - NTX-801 (Cbl-b inhibitor); 1µM
- **Flow Cytometry**
  - Performed on both T-cell cultures, spleen and tumor samples.
  - Cells were stained with fluorophore-conjugated antibodies recognizing lineage specific and functional markers.
  - Data were analyzed using flow cytometry with appropriate gating strategies.
- **Organoid Culture**
  - Tumor-derived organoids were cultured in Collagen-based media.
  - Treated with experimental compounds and stained with viability dyes to assess growth, viability, and immune signaling effects.
- ***In Vivo* Studies**
  - Immunocompetent female mice (FVB, 5-7 weeks old) were injected orthotopically with TNBC C0321 cells.
  - Mice received oral gavage of DHA (10mg/kg) or NTX-801 (30 mg/kg) suspended in HPMC (hydroxypropylmethylcellulose) daily.
  - Tumor progression and T-cell responses were evaluated via flow cytometry and morphological analysis.

Pharmacological inhibition of TRIM21 impacts T-cell function *in vitro.*

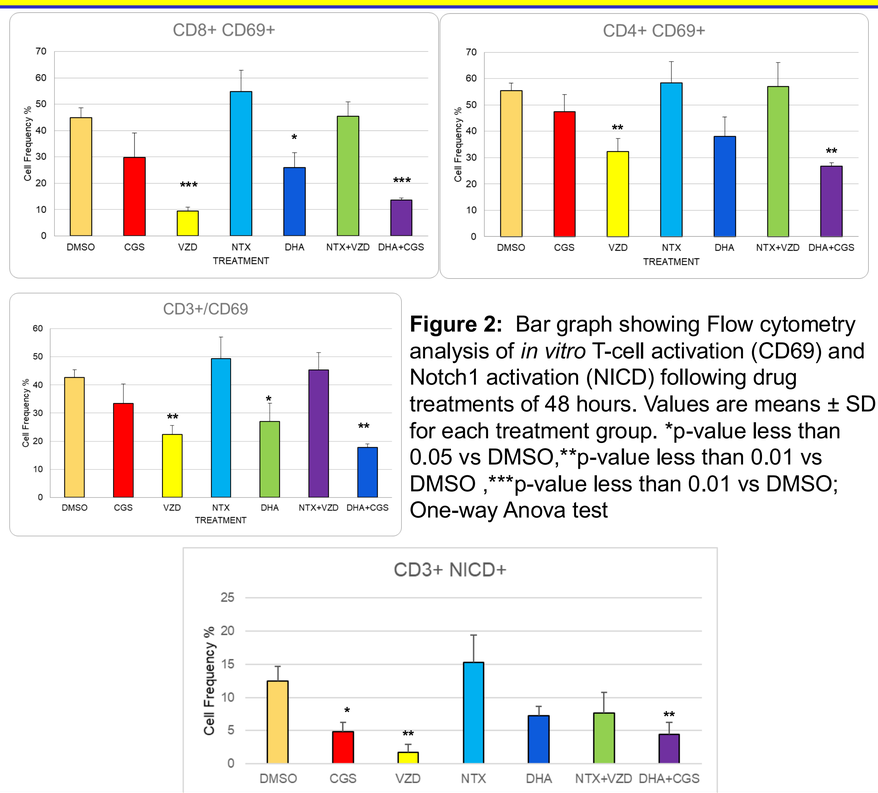

Figure 2: Bar graph showing Flow cytometry analysis of *in vitro* T-cell activation (CD69) and Notch1 activation (NICD) following drug treatments of 48 hours. Values are means ± SD for each treatment group. *p-value less than 0.05 vs DMSO,**p-value less than 0.01 vs DMSO ,***p-value less than 0.01 vs DMSO; One-way Anova test

Dihydroartemisinin inhibits T-cell activation and Notch1 expression in a TNBC mouse model.

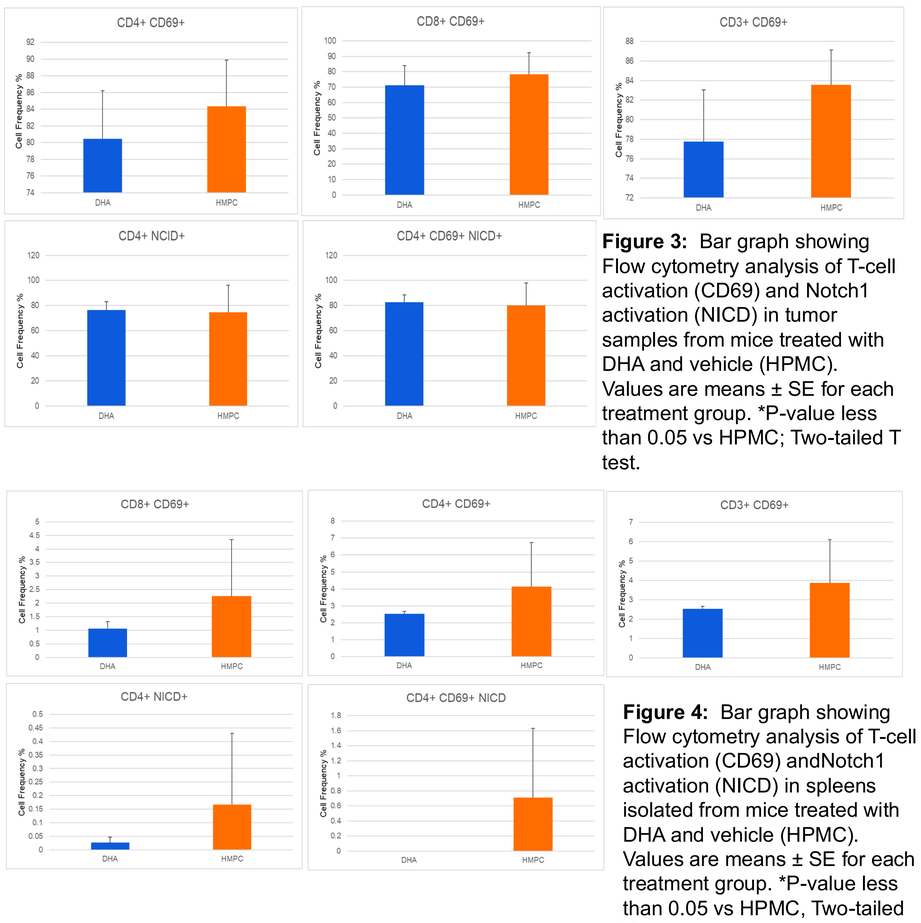

Figure 3: Bar graph showing Flow cytometry analysis of T-cell activation (CD69) and Notch1 activation (NICD) in tumor samples from mice treated with DHA and vehicle (HPMC). Values are means ± SE for each treatment group. *P-value less than 0.05 vs HPMC; Two-tailed T test.

Figure 4: Bar graph showing Flow cytometry analysis of T-cell activation (CD69) andNotch1 activation (NICD) in spleens isolated from mice treated with DHA and vehicle (HPMC). Values are means ± SE for each treatment group. *P-value less than 0.05 vs HPMC, Two-tailed T test.

Dihydroartemisinin increased tumor progression in a TNBC mouse model.

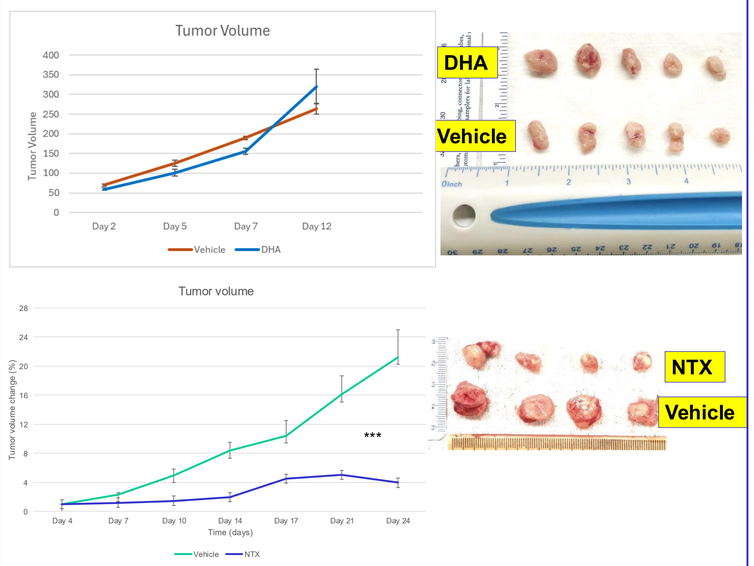

Figure 5: Tumor progression in a TNBC orthotopic mouse model (C0321) treated with DHA and vehicle over 12 days; NTX and vehicle over 24 days (previous study). ,***p-value less than 0.01 vs vehicle; one-way Anova

DHA and VZD increases growth of tumor-derived organoids.

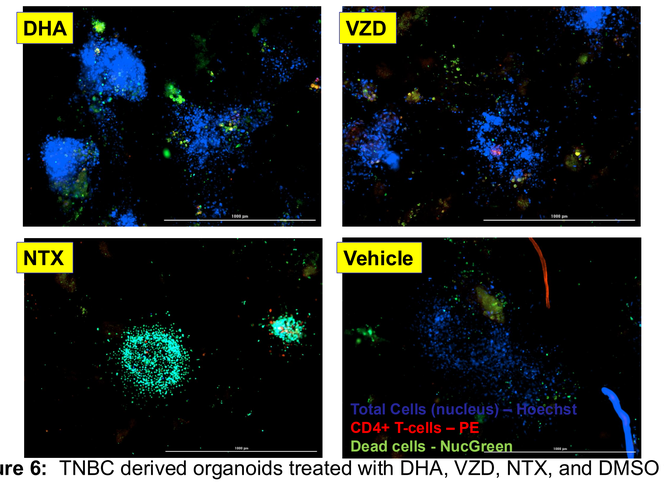

Figure 6: TNBC derived organoids treated with DHA, VZD, NTX, and DMSO for 6 days.

Conclusion

Our findings indicated that DHA, VZD, and CGS-21680 promote tumor progression via an immunosuppressive mechanism while NTX-801 enhances the anticancer immune response. Specifically, we found that DHA, CGS-21680 and VZD reduce proliferation and activation of T-cells as well as Notch1 expression while NTX-801 displayed an opposite effect in primary cell cultures. Furthermore, *in vivo* and *ex-vivo* studies revealed increased tumor progression and reduced T-cell responses in a TNBC mouse model treated with DHA which is opposite to the anticancer effect of NTX-801 observed in a previous study. These findings suggest that the TRIM21-Notch1 axis is a promising therapeutic target in the treatment of TNBC. Future studies will focus on identifying a viable anti-tumor drug agent

References

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