Immunomodulation of Tumor Microenvironment by Reversing Hypoxia

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Background

Triple-negative breast cancer (TNBC): aggressive subtype with poor outcomes, limited therapies.
Tumor hypoxia: reduces T-cell infiltration/function, contributes to immune resistance.
Current oxygenation strategies: promising in preclinical models but limited clinical use.
$NanO_2$: novel perfluorocarbon oxygen carrier; reverses hypoxia in preclinical models and patients with GBM.
Paclitaxel: exerts anticancer effects by inhibiting tumor cell division
PD-1 inhibitors: enhance T-cell mediated tumor killing by preventing immune exhaustion

Hypothesis: $NanO_2$ combined with chemotherapy and/or immunotherapy contributes to reversal of hypoxia in the tumor microenvironment (TME)

Methods

Experimental Design (AIM 1)[6]

Model: 115 female BALB/cJ mice with 4T1 tumors in mammary fat pad

PBS + Air (1×)	PBS + Air (5×)
$NanO_2$ + Air (1×)	$NanO_2$ + Air (5×)
PBS + O_2 (1×)	PBS + O_2 (5×)
$NanO_2$ + O_2 (1×)	$NanO_2$ + O_2 (5×)

Experimental Design (AIM 2)

Model: 70 female BALB/cJ mice with 4T1 tumors in mammary fat pad

IT + Air (28 days)	Chemo + Air (28 days)
IT + Chemo + Air (28 days)	$NanO_2$ + IT + O_2 (28 days)
$NanO_2$ + Chemo + O_2 (28 days)	$NanO_2$ + IT + Chemo + O_2 (28 days)

$NanO_2$ (12 mg/kg DDFPe, retro-orbital) ± O_2 exposure (90 min). Short-term = single dose; Long-term 5-day regimen
IT = Anti-PD-1 immunotherapy; Chemo = Paclitaxel

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Collaborators
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Results

Hypoxia

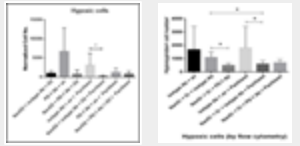


Fig. 1 and 2. Hypoxic Cells

T-Cell Activation

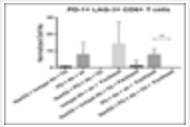


Fig. 3. CD8+ T Cells

Immune Checkpoints

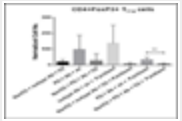


Fig. 4. Treg cells

Macrophages & TME Remodeling

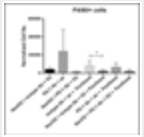


Fig. 5. Macrophages (F4/80+)

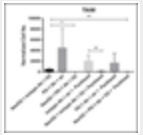


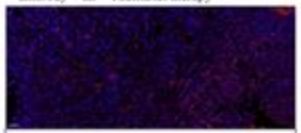
Fig. 6. Tumor Associated Macrophages



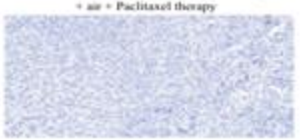
Hypoxyprobe immunofluorescence: isotype antibody + air + Paclitaxel therapy



PD-1 immunofluorescence: isotype antibody + air + Paclitaxel therapy



Hypoxyprobe immunofluorescence: NanO2 + PD-1 antibody + O2 + Paclitaxel therapy



PD-1 immunofluorescence: NanO2 + PD-1 antibody + O2 + Paclitaxel therapy

Discussion

Observed changes included:
↓ **PD-1 expression** and ↓ **T-cell exhaustion**.
↓ **Tregs** and ↓ **CD4+ T cells**, supporting reduced immune suppression.
↑ **TAMs (F4/80+, CD206+)**, consistent with TME remodeling.
↓ **Hypoxic cells** in combination with chemo and/or immunotherapy

Trends were consistent across most markers despite small sample size and variability.
Not all immune subsets showed significant changes (e.g., CD45+, CD11b+, granulocytes, B cells)

Conclusion

Paclitaxel + $NanO_2$ decreases tumor hypoxia in TNBC models.
PD-1 Ab + $NanO_2$ also was found to decrease tumor hypoxia in TNBC models.
LAG3+ exhausted CD8 T cells were decreased with $NanO_2$ + Paclitaxel with and without PD-1 Ab
It promotes proliferation of macrophages suggesting **macrophage remodeling** within the TME.

$NanO_2$ can potentially be used as an adjunct to chemotherapy to reverse hypoxia

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