# Immune Correlates Associated with Clinical Benefit in Patients with Immune Checkpoint Refractory HPV-associated Malignancies Treated with Triple Combination Immunotherapy

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## Abstract

**Background**
Globally more than 600,000 cases of HPV-associated cancers occur annually. Approximately 15-20% of cases respond to PD-(L)1 inhibitors, and approximately 30%, including 10% of immune checkpoint inhibitor (ICI) refractory patients, respond to bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1. Thus, for most patients who are ICI refractory, there is no effective therapy. Preclinical studies have shown that the triple combination of bintrafusp alfa, M9241, a tumor-targeting IL-12 immunocytokine, and PDS0101, a therapeutic vaccine targeting HPV-16, resulted in maximal tumor reduction. A phase II trial (NCT04287868) evaluating this triple therapy has shown a manageable safety profile and preliminary evidence of clinical activity in ICI refractory HPV-associated cancers, with 45% of patients having disease reduction, including 27% with objective responses.

**Methods**
Peripheral blood from patients with ICI refractory HPV-associated malignancies (n=27) treated with the triple therapy was analyzed prior to and 2 weeks post first treatment (a timepoint prior to restaging) for multiple serum cytokines and soluble factors, complete blood counts, and 158 immune cell subsets. HPV-16 specific T-cells were assessed before and during treatment in a subset of patients (n=14). Immune parameters were evaluated for changes with therapy and compared between patients deriving clinical benefit (with a best overall response of stable disease, partial response, or complete response) versus those with progressive disease (PD).

**Results**
The triple therapy promoted a pro-inflammatory serum cytokine and factor milieu, and significantly increased NK cells (p=0.002) and decreased conventional dendritic cells (cDCs, p=0.001), plasmacytoid DCs (p=0.011), CD4+ T-cells (p=0.008), CD8+ T-cells (p=0.004), T-regulatory-cells (p=0.002), and B-cells (p=0.042). HPV-16 specific T-cells were increased >2 fold after therapy in 11/14 patients evaluated. Before therapy, patients developing clinical benefit from the triple therapy had significantly higher levels of CD8+ naïve T-cells (p=0.037), trends of higher CD8:MDSC ratios (p=0.098), and significantly lower levels of cDCs (p=0.019) and classical monocytes (p=0.049), than patients developing PD. A greater early increase (2 weeks after one treatment cycle) in soluble granzyme B (p=0.004), TNFa (p=0.013), and monocytes (p=0.025), and less of a decrease in cDCs (p=0.006) associated positively with clinical benefit, while trends of an increase in the neutrophil to lymphocyte ratio (p=0.073) associated inversely.

**Conclusions**
These studies interrogating the peripheral immunome add insight into the combined mechanism of action of bintrafusp alfa, M9241, and PDS0101 in patients with HPV-associated cancers, and provide valuable information to identify ICI refractory patients potentially more likely to benefit from immunotherapy.

## Patient Demographics

| ICI Refractory (n=27) | n | % |
|---|---|---|
| **Age** | | |
| Median | 56 | - |
| Range | 37 - 81 | - |
| **Cancer Type** | | |
| Head & neck | 16 | 59% |
| Cervical | 6 | 22% |
| Anal | 4 | 15% |
| Rare | 1 | 4% |
| **HPV status** | | |
| HPV-16+ | 20 | 74% |
| HPV-16- | 7 | 26% |
| **Response** | | |
| **Responder (R)** | **9** | **33%** |
| R, HPV-16+ | 9 | - |
| R, HPV-16- | 0 | - |
| **Non-responder (NR)** | **18** | **67%** |
| NR, HPV-16+ | 11 | - |
| NR, HPV-16- | 7 | - |

**Figure 1. Summary of clinical characteristics and responses in immune checkpoint refractory patients treated on NCT04287868.** Responders (R) include patients with a best overall response (BOR) of complete or partial response (CR or PR, respectively), or stable disease (SD). Patients with progressive disease (PD) are classified as non-responders (NR).

## Results

### Development of pro-inflammatory immune signatures with triple therapy

| Immune Correlate | unit | Direction of change | D1 | D15 | p value | # > 25% | # < -25% |
|---|---|---|---|---|---|---|---|
| IFNγ | pg/ml | ↑ | 6.32 | 14.08 | 0.001 | **19 (79%)** | 4 (17%) |
| IL-10 | pg/ml | ↑ | 0.35 | 1.35 | <0.001 | **23 (96%)** | 1 (4%) |
| IL-6 | pg/ml | ↑ | 1.79 | 2.17 | 0.015 | **12 (50%)** | 4 (17%) |
| TNFa | pg/ml | ↑ | 2.42 | 3.11 | <0.001 | **14 (58%)** | 1 (4%) |
| granzyme B | pg/ml | ↑ | 6.31 | 8.77 | <0.001 | **16 (67%)** | 1 (4%) |
| sCD27 | pg/ml | ↑ | 213.94 | 249.35 | 0.001 | 9 (38%) | 1 (4%) |
| AMC | K/ul | ↑ | 0.56 | 0.71 | <0.001 | 11 (46%) | 0 (0%) |
| NK | % of PBMC | ↑ | 7.83 | 10.33 | 0.002 | 14 (67%) | 1 (5%) |
| TGFB1 | pg/ml | ↓ | 23730.11 | 472.95 | <0.001 | 1 (4%) | **23 (96%)** |
| CD4+ | % of PBMC | ↓ | 18.25 | 15.38 | 0.008 | 2 (10%) | 8 (38%) |
| CD8+ | % of PBMC | ↓ | 16.04 | 12.13 | 0.004 | 1 (5%) | 6 (29%) |
| cDC | % of PBMC | ↓ | 0.93 | 0.62 | 0.001 | 3 (14%) | **13 (62%)** |
| pDC | % of PBMC | ↓ | 0.13 | 0.10 | 0.011 | 3 (14%) | **12 (57%)** |
| Treg | % of PBMC | ↓ | 0.91 | 0.87 | 0.002 | 2 (10%) | **12 (57%)** |
| B cells | % of PBMC | ↓ | 9.02 | 8.27 | 0.042 | 5 (24%) | 9 (43%) |

**Figure 2. Changes in immune correlates at D15 compared to baseline that occurred with triple therapy.** Serum analytes (including cytokines and soluble factors), CBC measures, and peripheral immune subsets were measured at baseline and at Day 15 after triple therapy initiation. All markers in the table are significantly changed with therapy, by Wilcoxon paired statistical tests. Analytes where 50% or greater of patients had a greater than 25% increase or less than –25% decrease for each correlate are indicated in bold and underlined.

### Increased HPV-16 specific T cell responses with triple therapy

| Group | Developed HPV-16 T cell responses | Developed Multifunctional T cell Responses |
|---|---|---|
| All Patients | 11/14 (79%) | 7/14 (50%) |
| Responders (n=5) | 5/5 (100%) | 3/5 (60%) |
| Non-responders (n=9) | 6/9 (67%) | 4/9 (44%) |

**Figure 3. Development of HPV-16 specific T cells.** PBMCs were assessed at baseline and multiple timepoints post treatment for specific $CD4^+$ and $CD8^+$ T cells against HPV-16 E6 and E7 oncoproteins via *in vitro* stimulation with 15-mer peptide pools and assessed for the production of cytokines (TNFα, IFNγ, IL-2) and positivity for the degranulation marker CD107a by flow cytometry. Multifunctional T cells, positive for ≥2 measures, were also enumerated. A positive response was defined by a >2 fold increase over baseline at any time point evaluated. Both HPV-16+ and HPV-16- patients were included for HPV-specific T cell analyses.

### Immune correlates at baseline associated with clinical response

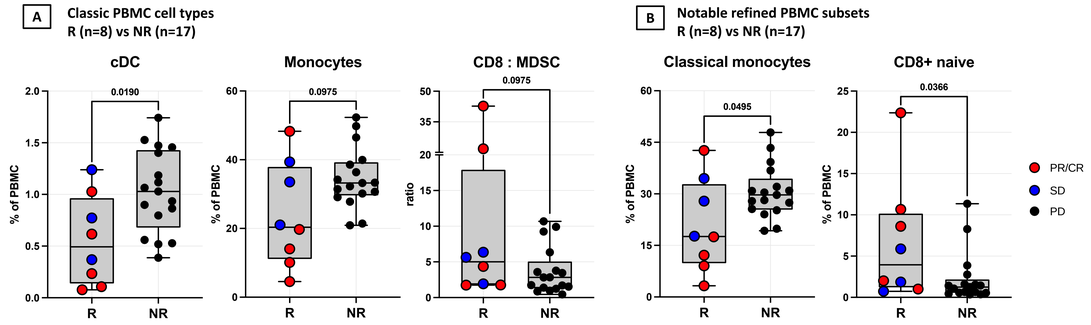


**Figure 4. PBMC immune subsets at baseline that are associated with clinical response with triple therapy.** Responders at baseline had **(A)** lower and trending lower frequencies of cDCs and monocytes, respectively, as well as a trending higher CD8:MDSC ratio. Responders at baseline also had **(B)** lower frequencies of classical monocytes and higher frequencies of CD8+ naïve T cells. Mann Whitney statistical tests were used to assess significance. BOR is indicated in the legend. No serum analytes or CBC measures at baseline were significantly associated with clinical response.

### Early changes at Day 15 in immune correlates associated with clinical response

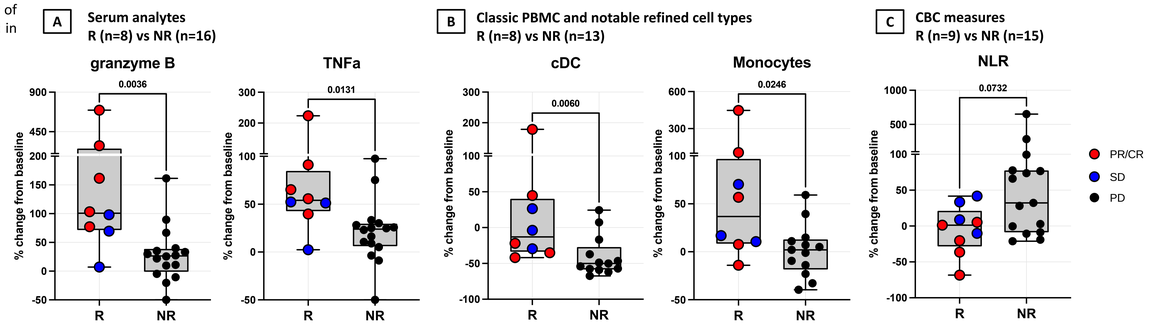


**Figure 5. Changes in immune correlates at Day 15 (timepoint preceding restaging) that are associated with clinical response to triple therapy.** **(A)** Responders had increased percent changes in granzyme B and TNFα compared to non-responders. **(B)** Responders had greater percent increases in cDCs and monocytes compared to non-responders. **(C)** Non-responders trended towards higher percent increases in neutrophil to lymphocyte ratios (NLR). Mann Whitney statistical tests were used to assess significance. BOR is indicated in the legend.

## Conclusions

- Treatment with triple therapy promotes a pro-inflammatory serum cytokine and factor milieu with increases in NK cells and monocytes, and decreases in dendritic cells and T cells. These changes highlight a role for triple therapy in the alteration in immunosuppressive forces, which could impact clinical outcomes.
- Immune profiling identified specific immune parameters *prior to therapy*, as well *as early changes* in specific immune parameters (at a time point preceding restaging) that may serve as predictive biomarkers to identify immune checkpoint refractory patients potentially more likely to benefit from immunotherapy.
- These data also support the continued evaluation of the triple combination of bintrafusp alfa (bifunctional fusion protein targeting TGF-β and PD-L1), M9241 (tumor-targeting IL-12 immunocytokine), and PDS0101 (therapeutic vaccine targeting HPV-16) in patients with immune checkpoint refractory HPV-associated malignancies.

## Ethics Approval

All patients gave written informed consent for participation in this clinical trial. This study was approved by the National Cancer Institute's Institutional Review Board. The trial registration number is NCT04287868.

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