**HPV-Negative HNSCC as an Immunotherapy Target**

HPV-negative head and neck squamous cell carcinoma represents a compelling target for IFN-gamma inducing immunotherapy, with emerging evidence revealing distinct molecular vulnerabilities and therapeutic opportunities despite inherent immune evasion mechanisms. **The convergence of high tobacco-induced mutational burden, reversible antigen presentation defects, and targetable resistance pathways creates a rational framework for combination immunotherapy strategies.**

**Molecular landscape drives neoantigen potential**

HPV-negative HNSCC exhibits a complex mutational architecture fundamentally shaped by tobacco carcinogenesis. Recent whole-genome analyses reveal **average tumor mutational burdens of 15.2 somatic exonic mutations**, with tobacco users showing significantly higher mutation rates than non-smokers (11.5 vs 5.6 mutations per patient). The tobacco-associated mutational landscape encompasses **six distinct signatures**, with SBS4 representing the predominant pattern characterized by **C>A transversions accounting for 22% of mutations**. This signature shows marked anatomical variation, appearing in 66.7% of laryngeal cases versus 17.3% of oral cavity tumors, suggesting site-specific carcinogen exposure patterns.

The neoantigen generation potential of tobacco signatures extends beyond simple mutational burden. **SBS4-positive tumors demonstrate 1.26-1.34 fold enrichment in stop-gain mutations**, creating protein-truncating events that generate novel tumor-specific antigens through frameshifts and altered protein sequences. These tobacco-signature mutations are enriched in early clonal events, indicating that carcinogenic exposure occurs in normal cells and contributes to neoantigen accumulation throughout tumor evolution. Combined with the finding that **tumors with TP53 and CDKN2A mutations show mean TMB values of 15.83-17.9 mutations per tumor**, the mutational landscape creates substantial immunogenic potential.

**p53 dysfunction amplifies both mutational burden and immune signaling disruption.** HPV-negative HNSCC shows **83.2% TP53 mutation frequency in smokers**, with these mutations creating a dual vulnerability: increased neoantigen load through enhanced mutagenesis and disrupted immune surveillance through interference with IFN-gamma signaling pathways. Specifically, mutant p53 interrupts the cGAS-STING pathway by preventing STING-TBK1-IRF3 complex formation and blocking IRF3-dependent type I interferon production, while also impairing MHC class I expression through disrupted STAT1 signaling.

**Antigen presentation machinery offers reversible therapeutic targets**

The immune evasion landscape in HNSCC reveals both challenges and opportunities for IFN-gamma-based interventions. **HLA class I loss occurs in 53% of HNSCC tumors**, exceeding previous estimates, with 15-25% of primary lesions showing total HLA class I expression loss. However, the critical finding is that **75% of HLA and antigen presentation machinery defects are non-structural and potentially reversible** through therapeutic intervention.

Antigen presentation machinery components show **coordinated downregulation in up to 70% of HNSCC lesions**, particularly affecting TAP1, LMP2, LMP7, and β2-microglobulin. The mechanisms underlying these defects include epigenetic silencing through DNA hypermethylation and histone deacetylation, EGFR-mediated MAPK pathway activation leading to transcriptional repression, and EZH2-mediated polycomb silencing of MHC class I genes. These mechanisms create therapeutic vulnerabilities addressable through multiple approaches.

**IFN-gamma represents the most potent inducer of antigen presentation machinery expression**, operating through JAK/STAT pathway activation to restore deficient APM component expression. Recent clinical evidence demonstrates the effectiveness of this approach: STING agonist MK-1454 achieved **24% objective response rates when combined with pembrolizumab**, compared to 0% as monotherapy. Epigenetic modulators show particular promise, with dual DNMT/HDAC inhibitors demonstrating superior efficacy compared to single agents, and **EZH2 inhibitor GSK126 enhancing both MHC class I expression and anti-PD-1 efficacy**.

**IFN-gamma pathway integrity determines therapeutic responsiveness**

The IFN-gamma signaling pathway in HNSCC shows complex patterns of dysfunction and therapeutic opportunity. While **JAK/STAT pathway components exhibit high mutation frequencies in therapy-resistant patients**, the majority of pathway defects appear to be functional rather than structural. SOCS proteins demonstrate **constitutive activation in many HNSCC cell lines**, suppressing interferon responses through JAK inhibition, while transcriptional suppression through AP2 and IRF2-mediated negative feedback creates additional resistance mechanisms.

**Epigenetic resistance mechanisms dominate over genetic alterations**, with DNA methylation of IFNGR, STAT1, and ISG promoters, chromatin remodeling defects affecting ISG accessibility, and SWI/SNF complex dysfunction suppressing IFN-gamma signaling. These mechanisms create therapeutic vulnerabilities, as **HDAC inhibitors enhance IFN-gamma pathway gene expression through chromatin remodeling** and increase MHC class I/II expression, while DNA methyltransferase inhibitors reverse methylation-mediated silencing of pathway genes.

**CDK4/6 inhibitors emerge as particularly promising IFN-gamma pathway enhancers**, operating through multiple mechanisms including enhanced T cell activation via NFAT dephosphorylation, increased tumor antigen presentation through MHC-I upregulation, selective suppression of regulatory T cell proliferation, and activation of endogenous retroviral elements stimulating type III interferons. Preclinical studies demonstrate **4-fold increases in IFN-gamma production by CD8+ T cells** with CDK4/6 inhibitor treatment, along with reduced T cell exhaustion markers and improved tumor infiltration.

**Clinical evidence supports combination strategies**

Clinical validation of IFN-gamma pathway targeting in HNSCC comes from multiple sources. **Phase I trials of nivolumab plus IFN-gamma achieved manageable toxicity profiles** with a recommended phase II dose of 50 mcg/m², notable lack of immune-related adverse events, and promising early efficacy signals warranting phase II evaluation. Gene expression signatures provide additional validation: **six-gene IFN-gamma signatures show up to 95% negative predictive value** for immunotherapy response, while T cell-inflamed gene expression profiles containing IFN-gamma-responsive genes demonstrate superior predictive accuracy (AUC 0.75 vs 0.65 for PD-L1 IHC).

**Combined biomarker approaches incorporating IFN-gamma pathway function show markedly improved predictive accuracy.** The TP-PR index combining TMB with PIK3CA, TP53, and ROS1 mutations achieves AUC 0.77 versus 0.56 for TMB alone, while high TP-PR patients demonstrate **median overall survival of 25 versus 8 months** compared to standard care. Clinical trials consistently show that **high TMB patients (≥10 mutations/Mb) achieve median overall survival of 25 versus 10 months** with immunotherapy, representing a 2.5-fold improvement that aligns with the mutational burden characteristics of tobacco-associated HPV-negative HNSCC.

**Immune microenvironment shapes therapeutic requirements**

HPV-negative HNSCC exhibits fundamentally different immune characteristics compared to HPV-positive disease, requiring distinct therapeutic approaches. These tumors demonstrate **"cold" immunosuppressive phenotypes with 2-3 fold higher MDSC infiltration** and increased M2-polarized macrophages secreting IL-10, TGF-β, and ARG1. **Lower overall T cell infiltration and reduced checkpoint expression** create challenges for single-agent checkpoint inhibitor approaches, with response rates of **8.3% in HPV-negative versus 12.0% in HPV-positive tumors**.

The spatial organization of immune cells differs markedly, with **greater physical separation between immune effector cells and tumor cells** and stromal barrier formation by immunosuppressive cells. However, this creates therapeutic opportunities for IFN-gamma-based interventions, which can **promote T cell infiltration and activation while inducing immunogenic cell death pathways**.

**Combination strategies targeting both immune activation and suppression reversal show particular promise** for HPV-negative disease. MDSC depletion through CXCR2 antagonists, PDE5 inhibitors, or STAT3 inhibitors combined with checkpoint blockade addresses the immunosuppressive milieu, while TAM repolarization using CSF1R inhibitors or CD40 agonists converts the microenvironment from immunosuppressive to immunosupportive.

**Biomarker strategies enable precision approaches**

The complex molecular landscape of HPV-negative HNSCC necessitates sophisticated biomarker strategies for optimal patient selection. **Current approaches combining PD-L1 CPS with TMB assessment show limited predictive accuracy**, with CPS providing predictive value in only 29% of FDA-approved trials. However, **incorporating tobacco signature analysis, p53 functional status, and IFN-gamma pathway gene expression provides complementary predictive information**.

**High TMB combined with functional p53 pathway status enhances immunotherapy response prediction**, while tobacco signature-positive tumors demonstrate higher neoantigen loads suitable for vaccination strategies. **IFN-gamma pathway gene expression signatures identify patients most likely to benefit from pathway restoration approaches**, creating opportunities for precision medicine approaches that match therapeutic interventions to molecular characteristics.

**Clinical translation and future directions**

The evidence supports a rational approach to targeting HPV-negative HNSCC with IFN-gamma inducing immunotherapy through several converging strategies. **Patient stratification should incorporate tobacco signature analysis to identify high neoantigen burden tumors**, TP53/CDKN2A mutation profiling to assess pathway integrity, and HLA/APM status evaluation to determine restoration requirements.

**Therapeutic combinations should focus on sequential or concurrent administration** of APM-restoring agents (epigenetic modulators, CDK4/6 inhibitors) followed by or combined with checkpoint inhibition and IFN-gamma pathway activation. The **75% reversibility rate of antigen presentation defects** provides strong rationale for this approach, while the high mutational burden ensures adequate neoantigen substrate for immune recognition.

Clinical development priorities include phase II trials of IFN-gamma plus checkpoint inhibitor combinations with biomarker-driven patient selection, optimal timing and sequencing studies of APM-restoring agents, and investigation of triple combination approaches targeting mutation burden (vaccination), antigen presentation (epigenetic modulators), and immune activation (checkpoint inhibitors) simultaneously.

The convergence of high mutational burden, reversible immune evasion mechanisms, and targetable resistance pathways positions HPV-negative HNSCC as a rational target for IFN-gamma inducing immunotherapy, with multiple validated approaches available for clinical translation and combination strategies that address the fundamental biological characteristics distinguishing these tumors from their HPV-positive counterparts.