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Intratumoral CD8⁺ T-cell differentiation is linked to durable HPV-specific immunity after neoadjuvant HPV vaccination with long-acting IL-7 and PD-1 blockade

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Article

Keywords:

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11474333/v1

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Additional Declarations:
There is **NO** Competing Interest.

Figures are available in the Supplementary Files section.

Intratumoral CD8$^+$ T-cell differentiation is linked to durable HPV-specific immunity after neoadjuvant HPV vaccination with long-acting IL-7 and PD-1 blockade

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Abstract: Durable antigen-specific immunity is a goal of therapeutic vaccination, but its relationship to intratumoral $CD8^+$ T-cell differentiation remains poorly defined. To address this question, we compared anti–PD-1, tirvalimogene teraplasmid (GX-188E), and long-acting IL-7 (GX-I7/NT-I7), alone and in combination, in HPV-positive tumor-bearing mice. Among tested regimens, triple immunotherapy produced the greatest tumor control and a pooled tumor profile enriched for stemness-associated intratumoral $CD8^+$ states. We then evaluated the same components in a four-cohort phase 2 neoadjuvant study of 56 patients with resectable HPV-positive head and neck squamous cell carcinoma. In the 11-patient cohort receiving pembrolizumab, GX-188E, and NT-I7, 7 patients had a major pathologic response, including 4 with a pathologic complete response. To define the intratumoral immune program accompanying this activity, paired tumor profiling identified a *GZMK*$^+$ effector-memory $CD8^+$ state with cytotoxicity- and memory-associated features. *GZMK*$^+$ effector-memory cells were prominent among T cells bearing clonotypes shared between post-treatment tumors and tumor-draining lymph nodes, consistent with intercompartmental recirculation. Higher tdLN *Myc* expression in both NT-I7-containing mouse profiles prompted regulatory analysis of the human *GZMK*$^+$ state, revealing treatment-associated higher *MYC* and lower *BATF* regulon specificity; *in silico IL7R* perturbation was consistent with an IL-7-associated reprogramming model. Post-treatment intratumoral *MYC*-associated and relative *MYC*/*BATF* regulon measures were related to HPV16 E6/E7-reactive activation-induced marker responses at 2 years; in eight patients with paired tumors, treatment-associated relative *MYC*/*BATF* changes were separately related to a 2-year peptide-responsive $CD8^+$ stem cell–like memory phenotype. Together, these findings associate intratumoral *GZMK*$^+$ effector-memory remodeling with HPV-reactive activation and memory phenotypes 2 years later.

Main

Therapeutic cancer vaccines aim to mobilize tumor-antigen-specific immunity. The success of mRNA vaccines against COVID-19 renewed interest in this approach, yet strong preclinical activity has not consistently translated into broad benefit in established solid tumors[1, 2]. The central challenge is not simply to generate antigen-reactive T cells, but to sustain their expansion, trafficking into tumors, effector function, and memory under chronic antigen exposure. Addressing this challenge calls for combination strategies that couple immediate antitumor activity to durable functional T-cell immunity[3]. In human papillomavirus (HPV)-associated cancers, the viral oncoproteins E6 and E7 provide persistent 'non-self' targets because they are constitutively expressed and required for oncogenesis[4]. E6/E7-directed therapeutic vaccination has shown antitumor activity in preclinical HPV-associated models[5], and an HPV16-targeted vaccine combined with nivolumab has provided an early clinical precedent (NCT02426892)[6]. HPV-positive head and neck squamous cell carcinoma (HNSCC) also harbors HPV-specific B-cell responses and functional stem-like PD-1$^+$ CD8$^+$T cells[7, 8]. Persistent viral antigens and measurable HPV-specific immunity therefore make HPV-positive HNSCC a tractable setting in which to relate intratumoral CD8$^+$T-cell differentiation to antigen-reactive immunity over time.

HPV-positive HNSCC is not only an instructive model of antigen-specific immunity but also a growing clinical problem. HPV accounts for nearly 5% of cancers worldwide and for most oropharyngeal cancers, and the incidence of HPV-positive HNSCC is rising in high-income countries[9, 10]. Definitive chemoradiation therapy can cause substantial acute and chronic toxicity[11], and although trans-oral surgery can support treatment de-intensification in selected patients, no generally accepted de-escalation standard has emerged[12, 13]. PD-1 blockade is active in recurrent or metastatic HNSCC, but meaningful responses remain limited to a subset of patients[14, 15]. Perioperative pembrolizumab is now a standard of care in resectable HNSCC following KEYNOTE-689 [16], yet pathologic tumor reduction was modest (major pathologic response [mPR] 9.4%; pathologic complete response [pCR] 3.0%) and p16-positive tumors comprised only ~4% of the trial population under restricted eligibility criteria. Extrapolating single-agent PD-1 blockade to HPV-positive resectable disease is therefore not straightforward, underscoring the need to strengthen both the generation and the persistence of antitumor immunity. In resectable disease, neoadjuvant treatment creates a window of opportunity to evaluate such immune strategies directly by comparing pretreatment and resection tumors,

examining regional lymph nodes at surgery, and following antigen-reactive immunity longitudinally[17, 18]. Therapeutic vaccination can prime and expand tumor antigen–specific T cells, but priming alone may not sustain the magnitude or differentiation quality of the response. This rationale motivated the addition of cytokine support to promote the survival, expansion, and persistence of vaccine-elicited T cells, alongside checkpoint blockade to relieve inhibitory signaling within the tumor. Tirvalimogene teraplasmid (GX-188E) is a therapeutic DNA vaccine encoding HPV16/18 E6 and E7 that has shown activity in HPV-associated disease, particularly in combination with PD-1 blockade[19, 20]. Building on this GX-188E-based regimen, we incorporated efineptakin alfa (GX-I7/NT-I7), a long-acting IL-7 selected to provide sustained T-cell support[21, 22], together with pembrolizumab to relieve PD-1-mediated inhibition. We therefore asked whether combining antigen-specific priming, IL-7-mediated support, and PD-1 blockade could remodel intratumoral $CD8^+$T-cell differentiation and connect that tissue response to persistent HPV-reactive immunity. We first tested this combination strategy in an HPV-positive syngeneic mouse tumor model, in which triple immunotherapy produced the greatest tumor control among the tested regimens and generated a stemness-enriched intratumoral $CD8^+$T-cell landscape. We then evaluated the same three components in a four-cohort phase 2 neoadjuvant study (GENUINE trial) that enrolled 56 patients with locally advanced, resectable HPV-positive HNSCC. In the first cohort of 11-patient cohort treated with pembrolizumab, GX-188E, and high-dose NT-I7 at 1,200 μg/kg, the full regimen yielded mPR, including pCR, and HPV16 E6/E7-reactive T-cell responses remained detectable 2 years after treatment. Treatment also promoted germinal center (GC)-like-B cell program and the formation of tertiary lymphoid structure (TLS). Within this local adaptive niche, the tumor-reactive $CD8^+$ compartment showed transcriptional reinvigoration and *STAT5*-associated memory rewiring. A $CD8^+$*GZMK*$^+$ effector-memory population sharing TCR clonotypes between tumors and tumor-draining lymph nodes (tdLNs) marked a clonotypically connected, transitional state with high cytotoxicity-associated scores and an intermediate differentiation profile. Human regulon analyses identified treatment-associated *MYC–BATF* rebalancing, while pooled mouse profiles and in silico IL7R perturbation supported an IL-7-associated reprogramming model for this state. These intratumoral regulatory features were further related to complementary HPV16 E6/E7-reactive activation and stem-cell-like memory phenotypes detected in peripheral blood 2 years later. Together, these findings suggest that early shaping of intratumoral $CD8^+$T-cell differentiation may determine the durability of vaccine-induced immunity.

Results

Triple immunotherapy couple tumor control with a stemness-enriched intratumoral CD8$^+$ T-cell landscape in mice

To define the antitumor and immunologic effects of anti–PD-1, the therapeutic HPV DNA vaccine (tirvalimogene teraplasmid; GX-188E), and long-acting IL-7 (efineptakin alfa; GX-I7/NT-I7), we compared these agents as single treatments and in dual- and triple-combination regimens in TC-1 tumor-bearing C57BL/6 mice (**Fig. 1a**). The triple regimen produced the greatest tumor regression among the tested regimens (**Fig. 1a** and **Extended Data Fig. 1a, b**) and the longest survival, with a significant difference across treatment groups (**Fig. 1c**). The intratumoral CD8$^+$ area measured by immunohistochemistry was also greatest with the triple regimen, with significant differences across regimens (**Fig. 1d** and **Extended Data Fig. 1c**). Because the strongest antitumor phenotype coincided with the greatest intratumoral CD8 representation, we next examined the CD8$^+$T-cell differentiation states associated with triple immunotherapy.

We performed single-cell RNA-sequencing (scRNA-seq) on group-level pooled tumors from five groups: untreated control, anti–PD-1, HPV vaccine plus anti–PD-1, HPV vaccine plus long-acting IL-7, and HPV vaccine plus anti–PD-1 plus long-acting IL-7 (**Fig. 1a** and **Extended Data Fig. 2**a). Subclustering of intratumoral CD8$^+$T cells resolved *Bach2*$^+$ naïve and *Sell*$^+$ central-memory (CM) populations; *Gzmm*$^+$ and *Klrb1c*$^+$ effector-memory (EM) populations; *Cd44*$^+$ and *Tnfrsf9*$^+$ activated populations; and exhausted (Tex), interferon-stimulated gene (ISG)-high, and proliferating populations (**Fig. 1e**). Whole-immune profiling placed these states within the broader intratumoral immune landscape (**Extended Data Fig. 2a, b**). Relative to anti–PD-1 alone and HPV vaccine plus anti–PD-1, the triple-regimen profile showed higher stemness-associated scores and lower exhaustion-associated scores (**Extended Data Fig. 2c**). Pseudotime ordering arranged the CD8$^+$T cells along a trajectory spanning four inferred transcriptional states (**Fig. 1f**). Cells from the anti–PD-1 and HPV vaccine plus anti–PD-1 groups were concentrated toward trajectory regions enriched for terminal-exhaustion signatures, whereas triple-regimen cells spanned a broader portion of the trajectory, with *Gzmm*$^+$ EM cells occupying intermediate positions. *Gzmm*$^+$ and *Klrb1c*$^+$ EM cells were most highly represented in the triple-regimen profile (**Fig. 1g**). Stemness scores declined and exhaustion scores increased across pseudotime in the anti–PD-1 and HPV vaccine plus anti–PD-1 groups, whereas stemness scores were

comparatively maintained in the triple-regimen group (**Fig. 1h**). Both NT-I7-containing arms showed higher *Myc* expression in tdLNs than the anti–PD-1 and HPV vaccine plus anti–PD-1 profiles, within a broader treatment-associated pattern of differentiation-related transcription factors across tumor and tdLNs compartments (**Extended Data Fig. 2d**). Collectively, these data established the biological premise for the human study. Among the tested regimens, triple immunotherapy achieved the strongest tumor control, and was accompanied in the pooled transcriptomic profile by a broader stemness-enriched intratumoral $CD8^+$T-cell distribution containing prominent *Gzmm*$^+$ and *Klrb1c*$^+$ EM states. Within this landscape, the higher tdLNs *Myc* signal shared by both IL-7-containing profiles provided an early regulatory clue to how IL-7 support might shape $CD8^+$T-cell differentiation, motivating evaluation of the same regimen in human tumors.

Neoadjuvant vaccine-based triple immunotherapy induces marked pathologic tumor regression

Guided by the mouse comparison of GX-188E, anti–PD-1, and NT-I7 alone and in combination, we conducted a phase 2 neoadjuvant study of pembrolizumab-, GX-188E-, and NT-I7-containing regimens in patients with locally advanced, resectable HPV16-positive HNSCC (NCT05286060; **Fig. 2a**). The study firstly enrolled cohort I (n = 11); subsequent patients were then randomized across the three prespecified expansion cohorts, II–IV (n = 15 each), for a total of 56 patients. Pembrolizumab 200mg was administered intravenously on days 1 and 22, GX-188E 2 mg intramuscularly on days 1, 8, and 22, and NT-I7 intramuscularly on day 8. Cohort I received pembrolizumab, GX-188E, and NT-I7 at 1,200 μg/kg; cohort II received pembrolizumab and GX-188E; cohort III received pembrolizumab, GX-188E, and lower-dose NT-I7 at 360 μg/kg; and cohort IV received GX-188E and NT-I7 at 1,200 μg/kg without pembrolizumab. After safety and activity had been assessed in cohort I, patients in cohorts II–IV were randomized among the three predefined regimens. The median age was 58 years (range, 37–73), and 50 of 56 patients (89.3%) were male (Table 1). Fifty-four tumors (96.4%) were HPV16-positive and two (3.6%) were HPV18-positive by nucleic acid testing; 46 patients (82.1%) had stage II disease and 10 (17.9%) had stage III disease. All 56 patients received their planned neoadjuvant regimen and were included in the safety and efficacy analyses.

We next evaluated safety and surgical feasibility across the four neoadjuvant regimens. All 11 patients in the cohort I experienced treatment-emergent adverse events (table S1). The most

common were injection-site reactions and maculopapular rash, each in 8 of 11 patients (72.7%), followed by elevations in aspartate and alanine aminotransferases, each in 7 of 11 (63.6%). Five patients (45.5%) had grade 3–4 events, including alanine aminotransferase elevation in 3 of 11, bilirubin elevation in 2 of 11, and an asymptomatic gamma-glutamyl transferase elevation in 1 of 11. Events in cohorts II–IV were predominantly grade 1–2; grade ≥3 aminotransferase abnormalities occurred in one patient in cohort II and two patients in cohort IV. Grade 1–2 maculopapular rash occurred in 11 of 15 patients in cohort III and 8 of 15 in cohort IV. Myocarditis occurred in one cohort III patient, and pneumonitis occurred in one cohort II patient. All 11 cohort I patients completed neoadjuvant therapy and underwent resection without treatment-related delay, at a median of 47 days after treatment initiation (range, 39–71 days). The corresponding median interval in cohorts II–IV was 70 days (range, 57–81 days), with no significant difference among the three cohorts.

With all 11 cohort I patients proceeding to resection on schedule, we evaluated the prespecified primary endpoint of mPR (mPR; ≤10% residual viable tumor). All 11 cohort I tumors showed pathologic regression, with a median pathologic tumor change of −95.0% (range, −100% to −15%). mPR was achieved in 7 of 11 patients (63.6%), exceeding the prespecified target rate of 35%; this total included 4 of 11 patients (36.4%) with pCR (pCR; no residual viable tumor in the resected primary tumor and sampled regional lymph nodes) (**Fig. 2b**). In cohorts II, III, and IV, mPR was observed in 4 of 15 patients (26.7%), 5 of 15 (33.3%), and 1 of 15 (6.7%), respectively (**Fig. 2b**). Radiologic response, evaluated in all 56 patients, provided complementary evidence of clinical activity. In cohort I, 7 of 11 patients achieved a partial response and the remaining four had stable disease, for an objective response rate (ORR) of 63.6%; objective responses occurred in 5 of 15 patients (33.3%) in cohort II, 4 of 15 (26.7%) in cohort III, and 1 of 15 (6.7%) in cohort IV (**Extended Data Fig. 3**). Four patients (two in cohort II and two in cohort IV) had disease progression before their planned resection. Three received definitive concurrent chemoradiotherapy (CCRT) instead of the planned surgery, whereas one underwent salvage resection (**Fig. 2b** and **Extended Data Fig. 3**). Adjuvant or definitive CCRT was administered to 5 of 15, 7 of 15, and 10 of 15 patients in cohorts II, III, and IV, respectively (**Fig. 2b**). In cohort I, two patients received adjuvant chemoradiotherapy for an abutted (≤1 mm) or close (≤5 mm) margin, whereas the other nine received no additional treatment. The full cohort I regimen therefore coupled on-schedule resection with marked pathologic regression.

HPV16 E6/E7-reactive T-cell responses remain detectable 2 years after neoadjuvant triple immunotherapy

All 11 cohort I tumors were HPV16-positive, providing a common antigenic basis for longitudinal monitoring of GX-188E-targeted HPV16 E6/E7 immunity. To track the kinetics and durability of this response, we analyzed serial peripheral blood mononuclear cell (PBMC) samples collected at baseline, cycle 2 day 1 (C2D1; D22), postoperatively (PostOP), and 2 years (2yr) after treatment. Cells were stimulated with overlapping peptide pools (OLPs) spanning HPV16 E6 and E7, and reactivity was measured as $CD69^{+}CD137^{+}$ cells among $CD8^{+}$T cells in activation-induced marker (AIM) assays, dual intracellular IFN-γ and TNF-α production, and antigen-induced IFN-γ release by ELISpot. Peptide-stimulated AIM reactivity was significantly higher than the paired antigen-negative control at C2D1 ($p = 0.0185$), postoperatively ($p = 0.0001$), and at 2 years ($p = 0.0379$), but not at baseline ($p = 0.1222$) (**Extended Data Fig. 5a**). Peptide-stimulated dual TNF-α^{+} IFN-γ^{+} production was significantly higher than the paired antigen-negative control postoperatively ($p = 0.0248$) and at 2 years ($p = 0.0178$), whereas the corresponding contrasts were not significant at baseline ($p = 0.4480$) or D22 ($p = 0.0645$) (**Fig. 2c, d**). IFN-γ ELISpot responses were numerically highest postoperatively and remained detectable at 2 years, with 10 evaluable samples at the 2-year time point (**Fig. 2e** and **Extended Data Fig. 5b, c**). Collectively, the full cohort I regimen showed coordinated clinical and immunologic activity, coupling marked pathologic regression and on-schedule resection with HPV16 E6/E7-reactive T-cell responses that emerged during or after treatment and persisted for 2 years.

Neoadjuvant triple immunotherapy promotes germinal center-like/tertiary lymphoid structure-associated adaptive immune organization

The clinical and immune activity observed with the full triple regimen in cohort I led us to examine how treatment remodeled the tumor immune microenvironment, using exploratory spatial and single-cell analyses of tumor tissue collected before treatment and at resection. AI-assisted spatial analysis of tumor-infiltrating lymphocytes (TILs) in paired pre- and post-treatment specimens from nine patients showed that, at baseline, six of nine tumors (66.7%) were immune-excluded, two (22.2%) were immune-desert, and one (11.1%) was immune-inflamed (**Fig. 2f**). Intratumoral and stromal TIL densities increased significantly after treatment (p=*0.00061, and p=0.0066 respectively*; **Extended Data Fig. 4a**), and the paired series shifted

toward an immune-inflamed phenotype. Thus, tumors that had been predominantly immune-excluded or immune-desert adopted a more inflamed tissue architecture at resection.

To resolve the cellular and molecular composition of this remodeled tissue, we performed scRNA-seq on pretreatment tumors (n = 10), post-treatment tumors (n = 11), and post-treatment tdLNs (n = 11), yielding 106,122 cells spanning 13 major populations (**Fig. 3a** and **Extended Data Fig. 7a–c**). Within the broad immune atlas, B cells were disproportionately represented in the treatment-remodeled context, with a higher relative abundance after treatment (**Extended Data Fig. 7d**). Post-treatment immune cells were also enriched for B-cell proliferation, B-cell receptor (BCR) /antigen-receptor signaling, and antigen-response programs (**Fig. 3b**).

Because the broad atlas highlighted this B-cell signal, we next subclustered this compartment into 10 states encompassing naïve, activated, germinal-center (GC) light- and dark-zone-like, memory, and plasma-cell phenotypes (**Fig. 3c** and **Extended Data Fig. 8**). Pseudotime analysis identified two inferred trajectories from naïve B cells, a GC-like Path 1 and an extra-follicular Path 2 (**Fig. 3c-e**) (20). Along Path 1, but not Path 2, a published computational HPV-specificity score increased with pseudotime (**Fig. 3f** and **Extended Data Fig. 9a**)[7]; within Path 1, this score was associated with GC-related scores and somatic hypermutation (SHM) (**Extended Data Fig. 9b**). The GC/extra-follicular (EF) signature-score ratio increased after treatment in tumors from patients who achieved mPR, including pCR ($p = 0.001$), but not in non-mPR tumors ($p = 0.8$; **Extended Data Fig. 9c**). Memory B cells positioned distally along Path 1 increased in relative representation after treatment, and showed higher GC signatures, BCR diversity, SMH, and HPV-specificity scores (**Fig. 3g–i** and **Extended Data Fig. 9d–g**). Together, these convergent B-cell features delineated a treatment-associated GC-like reaction with HPV-related features defined by published computational signatures.

AI-assisted spatial pathology analysis showed a marked increase in normalized TLS area score after treatment (**Fig. 3j, k** and **Extended Data Fig. 10**). At the patient level, post-treatment TLS area score correlated significantly with pathologic tumor regression ($p = 0.013$, $R^2 = 0.61$; **Fig. 3l**). Together, the shift toward spatial inflammation, the GC-like reaction, and the increased TLS area score defined a treatment-remodeled local adaptive niche, whose magnitude tracked with pathologic response.

Neoadjuvant triple immunotherapy reinvigorates and rewires tumor-reactive CD8$^+$ T cells

This GC-like, TLS-associated local adaptive niche established the context in which we next examined how the triple regimen remodeled intratumoral CD8$^+$ T cells. Subclustering of the tumor NK/T-cell compartment identified seven CD8$^+$ states: *BACH2*$^+$ naïve, *SELL*$^+$ central-memory, *GZMK*$^+$ effector-memory (*GZMK*$^+$-EM), TCF7$^+$ progenitor-exhausted-like (TPEX-like), *TOX*$^+$ exhausted-like (TEX-like), ISG-high, and *MKI67*$^+$ proliferating cells (**Fig. 4a** and **Extended Data Fig. 11a–c**). Signature analysis distinguished strong memory- and stemness-associated features in TPEX-like cells, cytotoxicity-associated features in *GZMK*$^+$-EM cells, and exhaustion-associated features in TEX-like and ISG-high cells (**Fig. 4b**). Expanded clonotypes were distributed across the *GZMK*$^+$-EM, TPEX-like, TEX-like, ISG-high, and proliferating populations (**Fig. 4c, d**), which also showed elevated *PDCD1* expression (**Extended Data Fig. 11c**). On the basis of clonal expansion and *PDCD1* enrichment, we operationally grouped these five states as tumor-reactive (TR) CD8$^+$T cells for downstream analyses.

After treatment, TR CD8$^+$T cells expressed higher levels of the memory- and stemness-associated genes *IL7R*, *CCR7*, and *TXNIP* and lower levels of the exhaustion-associated genes *TOX*, *CD244*, and *TIGIT* (**Fig. 4e**). Cytokine production, T cell activation, and immune-cell development pathways were also enriched after treatment (**Fig. 4f**). Patient-paired analysis confirmed a significant decrease in the exhaustion score and a significant increase in the memory/stemness score (**Fig. 4g**). At the state resolution, TEX-like cells showed lower expression of *TOX*, *TIGIT*, *LAG3*, and *HAVCR2* (**Fig. 4h** and **Extended Data Fig. 11d**). TPEX-like cells expressed higher *STAT5A*, a regulator implicated in T-cell rewiring during chronic antigen exposure[23], whereas TPEX-like and *GZMK*$^+$-EM cells showed higher *KLF2*, *SELL*, *BACH2*, and *ID3*. TPEX-like cells also expressed *CXCL13*, a chemokine associated with TLS-rich tumor microenvironments. The paired decrease in exhaustion-associated gene expression and score established transcriptional reinvigoration of the operationally defined TR compartment. In parallel, higher *STAT5A* expression in TPEX-like cells, together with the paired increase in memory/stemness score and concordant changes in TPEX-like and *GZMK*$^+$-EM cells, marked *STAT5*-associated memory rewiring.

We then related the post-treatment TR profile to relative intra-TLS area. Greater relative intra-TLS area trended toward a lower TEX/TR ratio ($p = 0.069$, $R^2 = 0.40$) and was significantly associated with a lower exhaustion score ($p = 0.043$, $R^2 = 0.46$; **Fig. 4i**). Thus, at the patient

level, greater relative intra-TLS area marked a lower exhaustion-signature profile in post-treatment TR cells. Among the TR states, *GZMK*$^+$-EM cells were distinguished by a high cytotoxicity-associated score while retaining memory-associated features, making this state a focus of subsequent analysis.

GZMK$^+$ effector-memory CD8$^+$ cells are prominent within tumor–draining lymph nodes shared clonotypes

Having identified *GZMK*$^+$-EM cells as a treatment-associated state of interest, we examined how their abundance and transcriptional features related to the marked responses in cohort I. *GZMK*$^+$-EM and TEX-like cells were well represented in tumors, and the proportion of *GZMK*$^+$-EM cells increased after treatment (**Fig. 5a, b**). At baseline, expanded *GZMK*$^+$-EM clonotypes were more frequently represented in tumors from patients who subsequently achieved mPR, including pCR, than in non-mPR tumors (**Fig. 5c**). *GZMK*$^+$-EM cells from the baseline tumors of these responders also showed higher expression of the activation- and interferon-response–associated genes *CD38*, *HLA-DRA*, *HLA-DPB1*, *HLA-DPA1*, *IFITM1*, and *IFNG* and of the tissue-homing chemokine receptor *CXCR3*, together with enrichment of T-cell activation and tissue-homing programs (**Fig. 5d** and **Extended Data Fig. 12a**). In post-treatment tumors, greater relative intra-TLS area was significantly associated with higher mean *CXCR5* expression in *GZMK*$^+$-EM cells ($p = 0.018$, $R^2 = 0.58$), whereas the *GZMK*$^+$-EM/TR ratio showed a positive trend with the intra-TLS count score ($p = 0.065$, $R^2 = 0.41$; **Fig. 5e**).

To test for cross-compartment clonotypic continuity, we compared TCR sequences across post-treatment tumors and tdLNs and detected identical *GZMK*$^+$-EM clonotypes in both tissues (**Fig. 5f**). Among the CD8$^+$ states, *GZMK*$^+$-EM cells contributed prominently to this cross-compartment overlap (**Fig. 5g**), and repertoire-level CDR3 tversky similarity also showed tumor–tdLNs relatedness (**Extended Data Fig. 12b**). Within tumors, clonotypes involving *GZMK*$^+$-EM cells were shared across multiple CD8$^+$ transcriptional states (**Fig. 5g**). Among the tumor-only, tdLNs-only, and tumor–tdLNs-shared clonotype categories evaluated, cells carrying shared clonotypes showed intermediate stemness/memory, tumor-reactivity, and exhaustion scores together with the highest cytotoxicity-associated score (**Fig. 5h**). Collectively. matching clonotypes and repertoire-level relatedness therefore identified a *GZMK*$^+$-EM state represented across the tumor and tdLN compartments, characterized by high cytotoxicity-associated transcriptional features and an intermediate differentiation profile.

MYC–BATF rebalancing marks IL-7-associated reprogramming of the GZMK$^+$ effector-memory CD8$^+$ state

To examine how the IL-7 adjuvant might contribute to this state, we returned to the higher tdLNs *Myc* signal observed in both long-acting IL-7-containing mouse profiles and asked whether a corresponding regulatory program was evident in human *GZMK*$^+$-EM cells. Using pySCENIC, we compared inferred regulon specificity score (RSS) values before and after treatment. RSS increased after treatment for *BACH2*, *MYC*, *MAFF*, and *KLF12* and decreased for *BATF*, *IRF9*, and *EOMES* (**Fig. 5i, j** and **Extended Data Fig. 12c**). In the plotted regulon space, *MYC* and *BATF* occupied distinct positions (**Fig. 5j**). The coordinated increase in *MYC* RSS and decrease in *BATF* RSS, summarized by the relative *MYC*/*BATF* RSS measure, defined treatment-associated *MYC–BATF* rebalancing within *GZMK*$^+$-EM cells. Among the evaluated regulons, *MYC*- and *BATF*-related measures showed the most consistent associations with higher TLS area score and greater pathologic tumor regression (**Fig. 5j** and **Extended Data Fig. 12d, e**). These human data identified *MYC–BATF* rebalancing as the principal treatment-associated regulatory feature of the *GZMK*$^+$-EM program.

To determine whether this human regulatory change was connected to the IL-7-containing component biology observed in mice, we reanalyzed arm-pooled murine CD8$^+$ scRNA/TCR-seq profiles from untreated control, anti–PD-1, GX-188E plus anti–PD-1, GX-188E plus NT-I7, and triple-regimen groups. Using clonal expansion and transcriptional features, we operationally distinguished bystander and tumor-reactive states (**Extended Data Fig. 13a, b**), and *Gzmm*$^+$-EM cells occupied an intermediate position along the inferred Slingshot trajectory (**Extended Data Fig. 13c**). Within this arm-pooled comparison, the triple-regimen profile showed the greatest tumor–tdLNs T-cell receptor overlap, with a prominent contribution from *Gzmm*$^+$-EM cells (**Extended Data Fig. 13d, e**). In parallel, Geneformer simulation of *IL7R* perturbation in human *GZMK*$^+$-EM cells and mouse *Gzmm*$^+$-EM cells predicted embedding shifts involving *MYC* and *BATF* (**Extended Data Fig. 12f, g**). *Myc*, *Batf*, and related memory- and differentiation-associated genes varied across the selected pooled treatment-arm profiles, and gene-program analysis showed state-level transcriptional correspondence between the murine *Gzmm*$^+$-EM state and the human *GZMK*$^+$-EM program (**Extended Data Fig. 13h-j**). Together, higher *MYC*-associated and lower *BATF*-associated RSS values after treatment in human *GZMK*$^+$-EM cells, higher tdLNs *Myc* in both NT-I7-containing pooled mouse profiles, cross-species *Gzmm*/*GZMK*

state correspondence, and *in silico IL7R* perturbation supported an IL-7-associated *MYC–BATF* reprogramming model. (**Fig. 5k**) This state combined strong cytotoxicity-associated features with retained memory-associated features and was represented among clonotypes shared by tumor and tdLN.

The *MYC–BATF* program in intratumoral GZMK$^+$ effector-memory CD8$^+$ is linked to HPV-reactive immunity 2 years later

To determine whether the post-treatment intratumoral *GZMK*$^+$-EM program was linked to long-term HPV16 E6/E7-reactive immunity, we used serial blood as a longitudinal readout after tumor removal. Postoperative AIM responses were associated with the post-treatment tumor *MYC* RSS (nominal $p = 0.045$) and relative *MYC*/*BATF* RSS measure (nominal $p = 0.048$), as were postoperative IFN-γ ELISpot responses (nominal $p = 0.013$ for both). At 2 years, the CD69$^+$CD137$^+$ AIM response was positively associated with *MYC* RSS (nominal $p = 0.004$), the relative *MYC*/*BATF* RSS measure (nominal $p = 0.007$), and the relative *TCF7*/*BATF* RSS measure (nominal $p = 0.019$). By contrast, the 2-year IFN-γ ELISpot response was not associated with *MYC* RSS (nominal $p = 0.495$), the relative *MYC*/*BATF* RSS measure (nominal $p = 0.668$), or the relative *TCF7*/*BATF* RSS measure (nominal $p = 0.539$; **Fig. 6a**). The association of the intratumoral *MYC–BATF* program with AIM responses therefore remained evident at 2 years, whereas its association with ELISpot did not.

Serial blood also contained a phenotypically defined peptide-responsive CD69$^+$CD137$^+$CD8$^+$ stem-cell-like memory T-cell (TSCM) subset detected after HPV16 E6/E7 stimulation (**Extended Data Fig. 14a, b**). At 2 years, its frequency was associated, across eight evaluable patient pairs, with the square-root-transformed tumor post/pre ratio of the relative *MYC*/*BATF* RSS measure ($R^2 = 0.6137$, $p = 0.0215$) and the relative *TCF7*/*BATF* RSS measure ($R^2 = 0.5343$, $p = 0.0394$; **Fig. 6b** and **Extended Data Fig. 14c–f**). Separately, overall circulating CD8$^+$TSCM frequency was associated with postoperative and 2-year IFN-γ ELISpot responses (nominal $p = 0.029$ and 0.037, respectively; **Fig. 6a**). Thus, treatment-associated rebalancing of the intratumoral *MYC–BATF* program was linked 2 years later to an HPV-reactive, peptide-responsive CD8$^+$TSCM phenotype.

Separately, as of the data cutoff on March 17, 2026, no patient in cohort I (n = 11) had experienced disease progression, recurrence, or death, after a median follow-up of 1,326 days (range, 1,281–1,385; **Fig. 6c**). Across the longitudinal immune analyses, *MYC–BATF*

reprogramming of post-treatment intratumoral *GZMK*$^{+}$-EM cells was linked to HPV16 E6/E7-reactive AIM responses and peptide-responsive CD8^{+}TSCM cells 2 years later.

Discussion

In this study, neoadjuvant triple immunotherapy remodeled intratumoral CD8^{+} differentiation toward a *GZMK*$^{+}$-EM program characterized by strong cytotoxicity-associated features with an intermediate differentiation profile, alongside GC-like, TLS-associated adaptive immune organization. Matching TCR clonotypes were detected among *GZMK*$^{+}$-EM cells were detected in post-treatment tumors and tdLNs, supporting a model of recirculation between the two compartments. Cross-species comparisons and computational analyses further pointed to an IL-7-associated *MYC–BATF* regulon model for this intratumoral state. Intratumoral *MYC–BATF* regulon features and their treatment-associated shifts were related to complementary HPV-reactive activation and memory phenotypes 2 years later. Together, these findings argue that durable therapeutic-vaccine immunity may depend not only on response magnitude of the response, but also on the differentiation quality of the responding CD8^{+} pool and its organization across the tumor–tdLNs axis.

Previous neoadjuvant checkpoint studies in resectable HNSCC established the value of examining tumors after a defined presurgical interval[16, 24, 25]. Tirvalimogene teraplasmid combined with PD-1 blockade also provided a therapeutic HPV-vaccine precedent in recurrent or advanced cervical cancer[19, 20, 26]. Building on these precedents, GENUINE used direct comparisons across mouse treatment arms to nominate the full combination, which produced the strongest tumor control among the tested arms. Selected single-cell profiles pooled by treatment arm showed a broader, stemness-enriched CD8^{+} landscape with the full regimen. The same three components were then evaluated in a 56-patient, four-cohort neoadjuvant study that included the full regimen, checkpoint–vaccine, lower-dose IL-7 triple, and vaccine–IL-7 regimens, with mPR observed in all four cohorts. We profiled the full-regimen cohort in depth: all 11 tumors regressed pathologically, 7 patients achieved mPR, including 4 pathologic complete responses, and all underwent resection without treatment-related delay. Paired pretreatment and resection tumors, surgical tdLNs, and serial blood then allowed the clinical activity of the regimen selected in mice to intratumoral differentiation, tumor–tdLNs clonal organization, and HPV-reactive immunity over 2 years.

Recent studies indicate that *GZMK* marks context-dependent CD8$^+$ T-cell states across the differentiation landscape rather than defining a fixed lineage. In neoadjuvant HNSCC, *GZMK* has been detected across effector-memory and activated or exhausted phenotypes and linked to recent tumor entry and differentiation[27]. An independent neoadjuvant HNSCC study placed *GZMK*-high effector-memory cells along earlier differentiation branches whose dynamics varied with treatment context[28]. After TCR stimulation, *GZMK* expression can decline as *GZMB*-associated, proliferative, and more differentiated effector programs emerge[29]. Clonally expanded *GZMK*$^+$ populations in inflamed human tissues further illustrate the context dependence[30]. The changes observed in GENUINE fit this emerging view: treatment shifted the tumor-reactive CD8$^+$ compartment toward greater memory/stemness and lower exhaustion, increased the proportion of *GZMK*$^+$ effector-memory cells, and enriched memory-associated regulators within this cytotoxicity-associated population. At baseline, expanded TCR clonotypes represented among *GZMK*$^+$ effector-memory cells were more prominent in tumors that later achieved mPR and within individual tumors, these clonotypes were also distributed across multiple other CD8$^+$ states. These observations place *GZMK*$^+$ effector-memory cells in an intermediate, treatment-responsive position along CD8$^+$ differentiation, where strong cytotoxicity-associated features coexist with memory/stemness features and response-associated clonal organization.

The treatment-associated shift in CD8$^+$ differentiation occurred alongside broader remodeling of the tumor microenvironment. Predominantly immune-excluded or immune-desert tumors adopted an inflamed architecture, while a GC-like reaction and increased TLS area score together characterized a treatment-remodeled local adaptive immune niche. HPV-related transcriptional features along the GC-like trajectory were consistent with prior direct evidence of HPV-specific intratumoral B-cell responses in HNSCC[7]. Post-treatment TLS area score was associated with pathologic regression, whereas greater relative intra-TLS area marked lower exhaustion among tumor-reactive CD8$^+$ cells. These findings accord with reports that mature TLS in HNSCC coordinate B-cell maturation with stem-like T-cell states and that functionally verified tumor-reactive clonotypes in renal cell carcinoma can be enriched in TLS and exhibit stem-like programs[31, 32]. TPEX-like cells expressed *CXCL13*, whereas *CXCR5* expression in *GZMK*$^+$-EM cells tracked with relative intra-TLS area. Together with external recruitment data, this complementary expression pattern raises the possibilty that *CXCL13–CXCR5* may help recruit and organize *GZMK*$^+$-EM during TLS-associated remodeling[33].

Clonal relationships also extended beyond the tumor: matching TCR clonotypes were detected among *GZMK*$^{+}$-EM cells in post-treatment tumors and tdLNs. Cells carrying TCR clonotypes shared between post-treatment tumors and tdLNs combined the highest relative cytotoxicity with intermediate memory/stemness and exhaustion. This cross-compartment pattern accords with clonally related progenitor-like cells in regional nodes and more differentiated states in tumors in HNSCC, as well as with preferential tissue–draining-node sharing of pathogen-specific, *GZMK*-biased clones in non-tumor human lung[34, 35]. We therefore propose a recirculation model in which *GZMK*$^{+}$ effector-memory cells form a clonally distributed cellular hub connecting TLS-associated immune organization in the tumor with a related pool in the tdLNs. Matching clonotypes do not establish cell movement or direction; however, mouse data raise the possibility that tdLNs replenish the intratumoral pool[36].

Vaccine adjuncts can shape both the magnitude of antigen-primed CD8^{+} expansion and the differentiation quality of the responding cells[37]. Long-acting IL-7 offers one such strategy, and NT-I7 served in this study as a systemic cytokine adjunct to vaccination. Its best-established human action is prolonged T-cell expansion, and recent pharmacodynamic data show that the first dose also induces proliferative and *MYC*-target programs[22, 38, 39]. In human *GZMK*$^{+}$ effector-memory cells, treatment increased *MYC*-associated regulon-specificity scores and decreased *BATF*-associated scores. Relative *MYC*/*BATF* measures were also related to TLS area score and pathologic regression. These measures describe relative regulon configuration rather than a biochemical *MYC*/*BATF* ratio or direct transcription-factor abundance, activity, or target occupancy. The direction of this regulon shift accords with the relatively higher *MYC* and lower *BATF* observed in human *IL7R*α-high effector-memory cells and within vitro IL-7 redirection of recently activated human CD8^{+} cells toward a recall-competent memory-like state[40, 41]. Mouse cancer-vaccine studies likewise support effects of co-encoded or NT-I7 on antigen-specific expansion, memory differentiation, persistence, and tumor control[42, 43]. Both selected pooled profiles from NT-I7-containing mouse arms showed higher tdLN *Myc*, and the murine *Gzmm*$^{+}$ effector-memory state showed transcriptional correspondence with the human *GZMK*$^{+}$ effector-memory program. Given the context-dependent functions of *BATF*, lower *BATF*-associated specificity is best understood within this *GZMK*$^{+}$-cell configuration rather than as a universally favorable change[44, 45]. These findings are consistent with a mechanistic model in which NT-I7 helps reprogram the *GZMK*$^{+}$ effector-memory state through a relative *MYC–BATF* shift that couples strong cytotoxicity-associated features with memory/stemness-associated features,

although itremains unresolved whether IL-7 redirects the fate of individual cells or preferentially expands a pre-existing configuration.

The post-treatment intratumoral *MYC–BATF* program was related to HPV-reactive immunity measured 2 years later. Neoadjuvant studies have connected tumor and blood clonotypes over early treatment intervals, whereas vaccine studies have documented multiyear antigen-specific persistence[46, 47]. HPV-directed cellular responses have also been followed for at least 1 year in HNSCC and 2 years in cervical disease[48, 49]. What remained unclear was whether a treatment-associated intratumoral differentiation program would relate to later antigen-reactive immunity in the same patient. HPV16 E6/E7-reactive responses remained detectable across complementary assays 2 years after therapy. Intratumoral *MYC*-associated and relative *MYC*/*BATF* measures were nominally related to 2-year AIM responses. In eight evaluable patient pairs, treatment-associated changes in the relative *MYC*/*BATF* and *TCF7*/*BATF* measures were separately related to a peptide-responsive $CD8^+$ stem-cell-like memory phenotype.

Corresponding relationships with 2-year IFN-γ ELISpot were not detected. This assay-specific pattern may reflect later activation and memory potential rather than uniform preservation of a single effector output, although assay sensitivity, small sample size, and exploratory testing provide alternative explanations. Because serial tumor tissue was unavailable, antigen-defined blood assays provided an accessible late readout of the earlier tissue biology. By relating intratumoral differentiation during treatment to later HPV-reactive activation and memory phenotypes in the same patients, these analyses add a temporal dimension beyond 2-year detectability alone.

Taken together, these results support a working model centered on a treatment-responsive *GZMK*$^+$ effector-memory pool that acts as a cellular hub across the tumor–tdLN axis, with IL-7-associated *MYC–BATF* reprogramming helps shape this pool and to link the intratumoral program to later HPV-reactive immunity. Several limitations temper these conclusions. Although the expansion cohorts (II–IV) were randomized concurrently under prespecified stratification, our deepest immune profiling centered on the initial, separately enrolled feasibility cohort (cohort I), and the overall sample was relatively small; the mouse single-cell analyses further relied on profiles pooled by treatment arm. Patient-level associations were exploratory and relied partly on computational inference of HPV-related B-cell features, AI-assisted quantification of the TLS area score, and inferred regulon specificity. The current data do not establish human

component-specific causality or mature TLS function, the antigen specificity of shared tumor–tdLN TCR clonotypes, the direction of *GZMK*$^{+}$-cell exchange between tumor and tdLN, or clonal continuity from tumor to later blood. Spatial TCR mapping combined with functional HPV-reactivity testing across tumor, TLS, tdLN, and serial blood could establish antigen specificity, define niche occupancy, and test long-term clonal continuity. Lineage tracing or photoconversion in matched models could then determine the direction of exchange. Factorial, time-resolved perturbation of *IL7R*, *MYC*, and *BATF*, coupled with direct measurements of protein activity, chromatin state, *GZMK*$^{+}$-cell fate, cytotoxicity, and recall, could distinguish active fate redirection from selective survival or expansion. Durable HPV-reactive recall may therefore be encoded before surgery in a CD8^{+} pool that is clonally connected across tumor and tdLN and displays both memory/stemness-associated and cytotoxicity-associated features.

Methods

Study design and patients

This was an investigator-initiated, phase 2 study of neoadjuvant triple-combination immunotherapy comprising pembrolizumab, tirvalimogene teraplasmid (GX-188E), and efineptakin alfa (GX-I7/NT-I7) in patients with resectable HPV-16- and/or HPV-18-positive HNSCC. An initial feasibility cohort (cohort I, n = 11) received the full triple regimen and was enrolled first; after protocol-specified safety review, three expansion cohorts (cohorts II–IV, n = 15 each) were opened, and patients were randomized across the three regimens using prespecified stratification factors including cancer stage (stage II/III vs stage IVA) and PD-L1 expression status (CPS ≥10 vs CPS <10) for a total of 56 patients. Each regimen differed in the investigational agents and/or the NT-I7 dose while maintaining the overall neoadjuvant strategy. The treatment schedule for each cohort was as follows (**Fig. 2a**): Cohort I (n = 11): pembrolizumab (200 mg) + GX-188E (2 mg) + high-dose NT-I7 (1,200 µg/kg), Cohort II (n = 15): pembrolizumab (200 mg) + GX-188E (2 mg), Cohort III (n = 15): pembrolizumab (200 mg) + GX-188E (2 mg) + low-dose NT-I7 (360 µg/kg), Cohort IV (n = 15): GX-188E (2 mg) + low-dose NT-I7 (360 µg/kg).

Pembrolizumab was administered intravenously on days 1 and 22, GX-188E intramuscularly with electroporation (EP02-MHC, Orbijector™) on days 1, 8, and 22, and NT-I7 by intramuscular injection on day 8 at the cohort-specific dose, before surgical resection. Surgery was planned within 2–8 weeks of treatment completion. Radiological evaluation by CT or MRI

was performed at baseline and after completion of neoadjuvant immunotherapy, before surgery. Eligible patients were aged ≥20 years with histologically confirmed, resectable stage II–III HPV-positive oropharyngeal HNSCC, as defined by the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. All 56 patients had measurable disease according to the Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1) and an Eastern Cooperative Oncology Group performance status of 0 or 1. HPV status was confirmed by p16 immunohistochemistry and HPV DNA genotyping. Pre-treatment tumor biopsies were obtained before initiation of neoadjuvant therapy, and post-treatment tumor specimens at surgical resection and were used for scRNA-seq and TCR-seq; peripheral blood was collected at protocol-defined time points for immunologic analyses up to 2 years after surgery.

The study was approved by the Severance Institutional Review Board (No. 4-2021-1786), all patients provided written informed consent before enrollment, and the trial was registered at ClinicalTrials.gov (NCT05286060) in March 2022. It was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Hypothesis

The study was designed with null and alternative hypotheses targeting an MPR rate of ≤5% and ≥35%, respectively. As part of the translational work to elucidate the mechanism of the triple combination therapy, baseline biopsy and resected tumor specimens at the time of surgery were collected for scRNA-seq and TCR-seq. The study was performed in accordance with relevant guidelines and regulations and adhered to the ethical standards of the institutional and national research committees.

Treatment outcomes

The primary endpoint was the MPR, defined 10% or less residual viable tumor in the surgically resected specimen. Secondary endpoints included safety assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, radiologic response based on RECIST v1.1, pathologic complete response (pCR, no residual viable tumor in the surgically resected specimen), locoregional recurrence-free survival (time from surgical resection to locoregional recurrence), distant recurrence-free survival (time from surgical resection to distant recurrence), recurrence-free survival (time from surgical resection to any type of recurrence), disease-free survival (time from surgical resection to locoregional recurrence,

distant recurrence, or death from any cause, whichever occurred first), and overall survival (time from treatment initiation to the date of death from any cause). The date cut off for this analysis was March 17, 2026.

Pathologic evaluation

Pretreated biopsy specimens underwent histopathological assessment to confirm the diagnosis and establish baseline tumor tissue morphology for comparison with any residual tumor after treatment. Pathologic tumor regression was evaluated from assessing the proportion of residual viable tumors in the post-treatment surgical specimens, together with documentation of tumor necrosis and regression. MPR and pCR were defined as ≤10% and 0% residual tumor viability, respectively, in the surgical specimen after neoadjuvant treatment. Pathologic tumor regression was defined as the sum of tumor necrosis and regression. PD-L1 expression was assessed by the PD-L1 IHC 22C3 pharmDx assay and expressed as a combined positive score (CPS). HPV status was confirmed by p16 immunohistochemistry and nucleic acid testing. Lunit SCOPE IO was used to analyze spatial TIL densities (intratumoral and stromal TILs) by detecting cells and segmenting tissue regions in hematoxylin and eosin–stained whole-slide images. Each whole-slide image, regardless of size, was divided into 0.25 mm^2 grids for analysis. The model estimated TIL densities and classified immune phenotypes based on the following criteria; 'inflamed,' defined as an intratumoral TIL density ≥130/mm^2; 'immune-excluded,' defined as an intratumoral TIL density <130/mm^2 and a stromal TIL density ≥260/mm^2; and 'immune-desert,' defined as TIL densities below the thresholds in both regions. The representative immune phenotype for each whole-slide image was determined as follows: 'inflamed,' when the proportion of grids with inflamed immune phenotypes was ≥33.3% of the whole-slide image; 'immune-excluded,' when the proportion of immune-excluded phenotypes was ≥33.3% and that of inflamed phenotypes was <33.3%; and otherwise classified as 'immune-desert.'

TLS area was quantified using Lunit SCOPE IO. The model identified lymphoid aggregates with areas of 12,000–240,000 μm^2. The TLS area score was calculated as total TLS area divided by total cancer area:

$$\textit{TLS area score} = \textit{total TLS area} / \textit{total cancer area}$$

The TLS burden was expressed as the intratumoral TLS area normalized to the cancer area (TLS area ratio) and as the intratumoral TLS cluster count normalized to the cancer area (TLS cluster

count ratio). Pre- and post-treatment values were compared in matched patients (**Fig. 3k** and **Extended Data Fig. 4b–d**), and the post-treatment TLS ratios were related to pathological tumor regression and to single-cell-derived measures (subset fractions, module scores and regulon activity ratios) by linear regression (**Fig. 3l, Fig. 4i, Fig. 5e**) or Spearman correlation (**Fig. 5j** and **Extended Data Fig. 12d, e**). Patients without evaluable paired slides were excluded, and the number of patients is given in each figure legend.

scRNA-seq processing

We used Cell Ranger v3.1.0 (10X Genomics) pipeline for FASTQ file generation from raw sequencing data, gene expression analysis of 5' gene expression library data, cell surface protein expression analysis of feature barcode library and immune profiling analysis of V(D)J enriched library data. Illumina basecall files from Illumina sequencing instrument were converted to FASTQ format for each sample using the 'mkfastq' command. Gene expression and feature barcode libraries were analyzed with the 'count' command. Sequencing reads of gene expression libraries were aligned to the GRCh38-1.2.0 genome reference using STAR (v2.5.1b) aligner. Those of feature barcoded libraries were matched to the target feature barcode reference. Next, gene and cell surface protein expression profiling in each cell was performed with the information of unique molecular identifier (UMI) and 10X cell barcode. Then, cells were grouped into clusters by their gene expression. V(D)J enriched libraries were analyzed with the 'vdj' command. Sequencing reads were assembled for each cell according to the unique molecular identifier (UMI) and 10X cell barcode. Then, clonotype calling was performed by matching assembled reads to the IMGT DB (GRCm38-alts-ensembl-3.1.0).

scRNA-seq data processing from clinical sample

Cell Ranger output files were read into R version 4.1.3 and processed using Seurat which allows the integrated processing of multi-modal (RNA, ADT, HTO) single cell datasets. As tumor tissue and draining lymph node in post-treatment samples are labelled with HTOs, cells for each dataset were first demultiplexed using a modified 2-steps HTODemux function in Seurat packages. Subsequently, the scRNA-seq data were consolidated into a unified Seurat object, and cells with low quality, meeting any one of the following criteria were excluded in the downstream analysis: (1) number of genes detected <300, (2) number of genes detected >9,000, and (3) fraction of mitochondrial genes >5%. Predicted doublets resolved on UMAP were removed at a later stage. The list of Seurat objects was created by splitting the dataset into

samples, and the following integration process was performed using Seurat Vignettes v4.3.0. Normalization and selection of variable features was conducted for each dataset with default parameters. The SelectIntegrationFeature function was then utilized to identify common variable genes across datasets, colloquially termed 'anchors'. Mitochondrial genes, ribosomal genes, T cell receptor (TRA, TRB), and immunoglobulin (IGL, IGK) genes were removed from the anchor gene set. Subsequent to the execution of the IntegrateData function, the list of Seurat objects was consolidated into a single object, and the scaling and the Principal Component Analysis (PCA) were conducted. Harmony was then applied after PCA and Uniform Manifold Approximation and Projection (UMAP) and clustering were performed on the "harmony space" to identify major clusters, with the aim of mitigating extra batch effects.

Murine scRNA and scTCR data processing

For murine scRNA and scTCR data processing, scRNA-seq data were processed with using Seurat as same with human processing 'mm10' assembly as the reference. Briefly, murine tumor and draining lymph node in each four different group are labelled with HTOs, cells for each dataset were first demultiplexed using a modified 2-steps HTODemux function in Seurat packages. Raw gene expression matrices were combined using the Seurat R package (version 4.3.1). We excluded HTO/ADT-featured cells with following criteria: (1) number of genes detected <200, (2) fraction of mitochondrial genes >10%. Normalization and selection of variable features was conducted for each dataset.

Each scRNA dataset from groups were normalized with Seurat by identifying reciprocal PCA with 30 dimensions for data integration of each group batches. Dimensionality reduction of the integrated matrix was performed using Uniform Manifold Approximation and Projection (UMAP) with the first 30 principal components. Phenotypic clusters were defined by constructing a k-nearest neighbors graph. After that, filtered genes were normalized by the NormalizeData function and the 4,000 variable genes were determined using the FindVariableFeatures function for cluster annotations. To reduce dimensionality for sub-clustering, the principal component analysis (PCA) was performed by RunPCA function. Cells were clustered using FindNeighbors and FindClusters consequence with different resolutions. The UMAP analysis was performed, and using FindMarkers function, the cluster genes were identified using 'MAST' test. For sub-clustering analysis of the lineage steps to clear $CD8^+$T cells, the count matrix of each sub-cluster was normalized and scaled and underwent

unsupervised clustering using PCs, as described above (T/NK sub-cluster: PCs = 20, resolution = 0.5; CD8 sub-cluster: PCs = 30, resolution = 0.2). Cell clusters in the resulting representation were annotated as known biological cell types using canonical marker genes.

The annotated murine CD8$^+$T-cell object was then used for the downstream analyses described above, namely signature scoring with AddModuleScore, TCR repertoire analysis with immunarch, pySCENIC regulon inference with the mm10 cisTarget databases and Geneformer in silico perturbation after mouse-to-human orthologue conversion. Mouse arms with fewer than 10 cells in the population analyzed were excluded from the regulon comparisons.

Cell type annotation and compositional analysis

Major immune lineages were annotated on the integrated object using canonical marker genes, which are shown as UMAP feature plots and as dot plots of the fraction of expressing cells and the scaled mean expression per lineage (**Extended Data Fig. 7a–c**). B cells and T/NK cells were re-embedded and sub-clustered separately, and B-cell and CD8$^+$T-cell subsets were annotated with the same marker-based approach (**Extended Data Fig. 8a, b** and **Extended Data Fig. 11a–c**). CD8$^+$T-cell subsets that are considered to contain tumor-reactive cells (CD8$^+$*GZMK*$^+$-EM, CD8-Tpex, CD8-Tex, CD8-ISG$^+$ and proliferating CD8) were additionally grouped as tumor-reactive (TR) CD8$^+$T cells and analyzed as one compartment where indicated.

For compositional analyses, the proportion of each lineage or subset was calculated per patient and time point as the number of cells of that population divided by the total number of cells of the parent compartment in the same sample, and the post-treatment to pre-treatment ratio of these proportions was computed per patient. Because the ratio is right-skewed and bounded at zero, it was square-root transformed for display and testing, and a ratio of 1 (no change) is indicated by a dashed line. Each population was compared with the overall mean of all populations in the same panel (**Fig. 5b and Extended Data Fig. 7d**). Patients without paired pre- and post-treatment samples for the compartment analyzed were excluded, and the number of patients contributing to each panel is given in the corresponding figure legend.

Differential gene expression and gene set enrichment analysis (GSEA)

Differentially expressed genes (DEGs) between two groups of cells were identified with the two-sided Wilcoxon rank-sum test implemented in Seurat's FindMarkers/FindAllMarkers functions on the RNA assay, using min.pct = 0.1 and logfc.threshold = 0.2 for the gene-ranking analyses. P

values were adjusted for multiple testing by Bonferroni correction over all genes in the dataset, as implemented in Seurat. In volcano plots, genes with an adjusted P value ≤0.01 and |log2 fold-change| >log2(1.3) were considered significantly deregulated; mitochondrial (MT-), ribosomal (RPS, RPL) and keratin (KRT) genes were not labelled, and functionally annotated genes were highlighted (**Fig. 4e, Fig. 5d**).

For GSEA, genes were ranked by the average log2 fold-change obtained from the same FindMarkers comparison after removing mitochondrial, ribosomal, keratin and immunoglobulin (IGL, IGK, IGH) genes and retaining genes with a nominal P ≤0.01. The ranked list was tested against Gene Ontology Biological Process (GOBP) gene sets with fgsea (v1.20.0; seed = 42, eps = 0, nPermSimple = 10,000, minSize = 3, maxSize = 500). Pathways were filtered to immune- or T-cell-related terms with the pattern lists used throughout the study, and the normalized enrichment score (NES) is shown for pathways passing the significance cut-off given in each figure legend (nominal p ≤0.05 in **Fig. 3b**, **Fig. 4f**; nominal p ≤0.01 in **Extended Data Fig. 12a**).

B cell trajectory analysis

Pseudotime trajectory analysis of the B cell compartment was performed with Monocle3 (v1.3.4). Two trajectories were learned from the same B cell embedding, one anchored on the germinal-center (GC) branch and one on the extrafollicular (EF) branch, and the naïve B subset was designated as the root population using the get_correct_root_state() function. Each cell was then assigned to Path1 (GC-like, cells only on the GC trajectory), Path2 (EF-like, cells only on the EF trajectory), Mixed (cells present on both trajectories) or None (cells on neither) (**Fig. 3c**). Downstream analyses of the B cell branches were restricted to Path1 and Path2 cells and, unless stated otherwise, to tumor samples (post-treatment lymph node excluded). Cell-type composition along pseudotime was visualized as a density of B-cell subsets scaled to the total density at each pseudotime value within Path1 and Path2 (**Fig. 3g** and **Extended Data Fig. 9d**), and the association of module scores with pseudotime was assessed by linear regression fitted separately in each branch (**Fig. 3f**).

Gene signature scoring

To characterize B cells, we utilized previously reported gene signatures for the germinal center (GC) reaction and extrafollicular (EF) reaction, including only genes with |log2FC| >1 and adjusted p-value ≤0.05 from the reference. Additionally, differential expression analysis was

conducted using DESeq2 (v1.34.0) based on data from Wieland et al. (Nat, 2020) to identify HPV-specific and tumor-infiltrating B cell signatures. Comparisons were performed between HPV-positive sorted tumor-infiltrating B cells and flu-specific B cells, as well as between HPV-positive sorted TIL B cells and naïve B cells. From the intersecting results, only genes with log2FC >1 and adjusted p-value ≤0.05 were selected as HPV-specific signature genes.

$CD8^+$T cells were characterized using gene signatures associated with stemness and memory phenotype (Galletti et al., Nat. Immunol., 2020), cytotoxicity (Galletti et al., Nat. Immunol., 2020), tumor reactivity (Oliveira et al., Nature, 2021), anti-PD-1 reactivity, progenitor exhaustion (Tpex) and exhaustion (Beltra et al., Immunity, 2020), and effective IFN-γ response and ISG-associated resistance (Benci et al., Cell, 2019).

All signature scores were calculated at the single-cell level with the AddModuleScore function in Seurat on the RNA assay. Because AddModuleScore returns values centered on zero, the scores that enter a ratio were shifted by the global minimum of the scores in that analysis before the ratio was taken. Specifically, the GC/EF ratio of a B cell was defined as (GC score − min) / (EF score − min + 0.001) and is displayed on a log2 scale, and the HPV specificity score was shifted in the same way before log2 transformation (**Fig. 3d–f, h** and **Extended Data Fig. 9**). For the IFN-γ signatures of $CD8^+$T cells, the difference between the two z-scored signatures of the effective IFN-γ and resistant ISG scores is shown in **Extended Data Fig. 11e**. For patient-level comparisons, single-cell scores were averaged per patient, tissue and subset, so that the unit of analysis is the sample rather than the cell; panels using single cells as the unit of analysis are indicated as such in the figure legends.

scBCR-repertoire analysis

scBCR-seq data was firstly pre-processed by Cell Ranger for V(D)J sequence assembly and BCR reconstruction using the GRCh38/hg38 assembly as the reference. The Change-O repertoire clonal assignment toolkit was used to define clones and we calculated BCR clonal abundances using tl.clone_size functions in dandelion (v0.3.5). We quantitatively assessed the similarity between BCR repertoires among groups based on the nucleotide sequences of the CDR3 regions. To identify SHM, BCR germline sequences were reconstructed using CreateGermlines.py, and the number of somatic mutations for each sequence was calculated using observedMutations (regionDefinition = IMGT_VDJ) (Shazam v1.2.0). Subsequent analysis is conducted with paired scBCR-seq data integrated with scRNA-seq data based on matched cell barcodes.

For the downstream analyses, each BCR sequence was annotated with the B-cell subset, trajectory branch (Path1/Path2), tissue and class-switch status (Sw/UnSw) of its cell. The somatic hypermutation frequency of B_AtM cells within Path1 was compared between pre- and post-treatment tumor separately for switched and unswitched sequences, with the BCR sequence as the unit of analysis (**Fig. 3i**). BCR clonal diversity was quantified as the inverse Simpson index of the clone-size distribution with the diversity function of vegan, computed for every combination of patient, class-switch status, tissue and B-cell subset within Path1; infinite values (samples with a single clone) were discarded and the index was square-root transformed for display and testing (**Extended Data Fig. 9f**).

scTCR-repertoire analysis

Cell Ranger outputs from scTCR-seq were processed in R (v4.1.3) using the immunarch package (v.0.9.1). TCRs were grouped into patient-specific TCR clonotype families based on TCRα-TCRβ chain identity, with cells sharing identical CDR3αβ amino acid sequences defined as belonging to the same TCR clone. The cell types of T cell subsets were identified by matching cell barcodes with scRNA-seq data and subsequently used in downstream analyses. Clonotypes with ambiguous CDR3αβ pairs (NA or missing chain) were excluded from all repertoire analyses.

To evaluate clonal expansion, the number of cells per clonotype was counted and clonality was categorized into three groups: single, double, and more than three cells per clonotype (**Fig. 4d**). Expanded clonotypes were defined per tissue and response group by summing the clone size of each CDR3αβ across patients of that group; the five largest clonotypes (Top5) and the next five (Top10, ranks 6–10) were mapped back to single cells by cell barcode and visualized on the UMAP, and the number of cells belonging to these clonotypes was counted per tissue and response group (**Fig. 4c, Fig. 5c**).

Clonotype sharing between the post-treatment tumor and the post-treatment lymph node was defined as CDR3αβ sequences detected in both tissues of the same patient. The number of unique shared clonotypes was counted per $CD8^+$T-cell subset and visualized as a stacked bar plot and as a chord diagram drawn with circlize (v0.4.16), in which the ribbon width is proportional to the number of shared clonotypes connecting each subset–tissue sector (**Fig. 5f**). Sharing between $CD8^+$T-cell subsets within post-treatment tumors was analyzed for clonotypes detected in at least three subsets and displayed as a network built with igraph (v2.0.3); node size is

proportional to the number of clonotypes detected in that subset and edge width to the number of clonotypes shared by the two connected subsets, with a fixed Fruchterman–Reingold layout (**Fig. 5g**). Repertoire similarity between subsets of the post-treatment tumor and the post-treatment lymph node of the same patient was computed with the repOverlap function of immunarch using the Tversky index, and only cross-tissue, within-patient subset pairs were retained (**Extended Data Fig. 12b**). For the clonotype-sharing phenotype analysis, CD8$^+$*GZMK*$^+$-EM cells of the post-treatment samples were labelled according to the tissue distribution of their clonotype as LN only, Tumor–LN shared or Tumor only, and signature scores were compared between these three groups at the single-cell level (**Fig. 5h**).

SCENIC regulon analysis

Gene regulatory networks were inferred with pySCENIC. Raw counts were exported from the CD8$^+$T-cell object to a loom file after removing genes detected in fewer than 1% of cells. Because GRNBoost2 is stochastic, the grn and ctx steps were repeated ten times with different seeds (seed = i × 100 + 7 for run i), using the hg38 (human) or mm10 (mouse) v10-clust cisTarget databases (10 kbp up/10 kbp down and 500 bp up/100 bp down gene-versus-motif rankings) together with the corresponding motif-to-TF annotation table and TF list. A consensus regulon set was then defined as follows: a regulon (TF) was retained if it was recovered in at least 8 of the 10 runs, a target gene was retained if it was found in at least 50% of the runs in which that regulon was recovered, and regulons with fewer than 10 stable target genes were discarded. Cells were scored for the consensus regulons with AUCell, yielding a cell-by-regulon AUC matrix.

In the human data, a regulon was considered active in a cell if its AUC exceeded the 90th percentile of that regulon across all CD8$^+$T cells, and the active-cell fraction was computed per cell type and time point. For CD8$^+$*GZMK*$^+$-EM cells, the log2 ratio of the active-cell fraction in post- versus pre-treatment tumor was plotted for the transcription factors most affected by in silico deletion of IL7R (see below), ordered by this ratio, together with the mean regulon AUC in CD8$^+$*GZMK*$^+$-EM cells (**Fig. 5i**, top 21 transcription factors; **Extended Data Fig. 12c**, top 50). For the association with clinical variables, the median regulon AUC was calculated per patient, cell type and time point, and the post/pre ratio of this value was computed for CD8$^+$*GZMK*$^+$-EM cells of each patient. This ratio was correlated with the post-treatment intratumoral TLS area ratio and with pathological tumor regression by Spearman rank correlation; transcription factors

with a nominal P ≤0.05 for at least one variable and belonging to the 50 most perturbation-sensitive transcription factors are displayed (**Fig. 5j and Extended Data Fig. 12d, e**).

In the mouse data, regulon specificity scores (RSS) were computed per treatment arm with the regulon_specificity_scores function of pySCENIC, restricted to CD8$^+$*Gzmm*$^+$-EM cells and to arms with at least 10 cells, and were z-scored within each arm across all consensus regulons before *Batf* and *Myc* were compared (**Fig. 5k**). In addition, the log2 ratio of the mean *Myc* to the mean *Batf* regulon AUC was calculated per treatment arm for all CD8$^+$T cells and for CD8$^+$*Gzmm*$^+$-EM cells, again excluding arms with fewer than 10 cells (**Extended Data Fig. 13f, g**).

Geneformer *in silico* perturbation

In silico perturbation was performed with Geneformer (V2, Geneformer-V2-104M_CLcancer). Raw counts of the human CD8$^+$T-cell object were tokenized with TranscriptomeTokenizer (model_version = "V2"), retaining the cell-type, patient, tissue and response annotations as custom attributes. For the mouse data, gene symbols were first converted to human Ensembl gene IDs using Ensembl BioMart mouse-to-human orthologues restricted to one-to-one relationships; genes without a one-to-one orthologue were discarded rather than mapped ambiguously, and the resulting matrix was tokenized in the same way.

IL7R (ENSG00000168685) was deleted in silico with InSilicoPerturber (perturb_type = "delete", combos = 0, model_type = "Pretrained", emb_mode = "cls_and_gene", cell_emb_style = "mean_pool", emb_layer = −1, forward_batch_size = 32), restricting the analysis to CD8$^+$*GZMK*$^+$-EM cells (human) or CD8$^+$*Gzmm*$^+$-EM cells (mouse) with the filter_data option and using all available cells. Gene-level effects were aggregated with InSilicoPerturberStats (mode = "aggregate_gene_shifts"), which reports, for every affected gene, the mean cosine similarity between its embedding in the perturbed and the unperturbed state together with the number of cells in which the gene was detected. The cosine distance was defined as (1 − mean cosine similarity).

Two filters were applied to the resulting table. For the plots of cosine distance against detection (**Extended Data Fig. 12f, g**), genes were restricted to the top 40% by number of detections and to the lowest 40% by mean cosine similarity, and only genes with a valid gene symbol were kept. For the transcription-factor rankings used in the SCENIC analyses, genes detected in fewer than

max(30, 4% of perturbed cells) cells, genes with zero variance across cells, and mitochondrial and ribosomal genes were removed, and the remaining genes were ordered by cosine distance; the 21 and 50 most affected transcription factors define the sets shown in **Fig. 5i** and **Extended Data Fig. 12c**, respectively.

Activation-induced marker assay

PBMCs (5 × 105 cells per well) were cultured for 16 h in a 1:1 mixture of AIM-V medium (Gibco) and RPMI 1640 (Welgene) in the presence of HPV16 E6/E7 overlapping peptide pools (2 μg/mL per peptide), 1 μg/mL anti-human CD28 and CD49d mAbs (BD Biosciences). Cells were subsequently stained with the LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Invitrogen), followed by surface staining with antibodies against CD3 (clone SK7; 1:50), CD4 (clone RPA-T4; 1:50), CD8 (clone RPA-T8; 1:50), CD69 (clone FN50; 1:25), and CD137 (clone 4B4-1; 1:25). Anti-CD14 and anti-CD19 antibodies were included as a dump channel to exclude monocytes and B cells, respectively. Data were acquired on a CytoFLEX LX flow cytometer (Beckman Coulter) and analyzed using FlowJo v10.8.1.

Flow cytometry

For intracellular cytokine staining (ICS), PBMCs (5 × 105 cells per well) were stimulated with HPV16 E6/E7 overlapping peptide pools (2 μg/mL per peptide) in the presence of anti-human CD28 and CD49d mAbs (1 μg/mL) for 48 h. Brefeldin A (10 μg/mL; Sigma-Aldrich) was added during the final 6 h of culture. Cells were stained with the LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Invitrogen) and antibodies against CD3 (clone SK7; 1:50), CD4 (clone OKT4; 1:50), CD8 (clone RPA-T8; 1:50). Anti-CD14 and anti-CD19 antibodies were included as a dump channel to exclude monocytes and B cells, respectively. For T-cell differentiation analysis, cells were additionally stained with antibodies against CCR7 (clone G043H7; 1:25), CD45RA (clone HI100; 1:25), CD95 (clone DX2, 1:25), and CD127 (clone A019D5; 1:25). Cells were then fixed and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) and stained intracellularly with anti-IFN-γ (clone 4S.B3; 1:25) and anti-TNF-α (clone MAb11; 1:25). Samples were acquired on a CytoFLEX LX flow cytometer (Beckman Coulter), and data were analyzed using FlowJo v10.8.1.

***Ex vivo* IFN-γ ELISPOT assay**

PBMCs (5 × 105 cells per well) were stimulated with 8 μg/mL of HPV16 E6 and E7 OLPs (PepTivator®, HPV16 E6 and HPV16 E7 respectively; Miltenyi Biotec) for 48 h. Phytohaemagglutinin and the medium only served as positive and negative controls, respectively. IFN-γ ELISpot assays were performed using Human IFN-γ ImmunoSpot kits (Cellular Technology Limited, Cleveland, OH, USA) according to the manufacturer's instructions. Spots were quantified using an automated ImmunoSpot Analyzer (Cellular Technology Limited) and expressed as spot-forming units (SFU) per 106 PBMCs after subtraction of background counts.

Animals

The experimental design for evaluating the efficacy of combinatorial treatments in tumor-bearing mice is detailed in **Fig. 1**. Briefly, C57BL/6 female mice were implanted 5 × 105 human E6/E7-expressing TC-1 tumor cells through subcutaneous, and treated with GX-188E using electrophoresis system (47 V, 10 ms) via intramuscular (I.M.). And NT-I7, and anti-PD-1 inhibitor, administered via I.M., and intraperitoneal (I.P.) injections, respectively.

Treatments were given at designated time points (Days 0, 1, and 7), followed by analyses at early, middle, and late stages (Days 9, 16, and 24, respectively). The timeline includes multiple stages of tumor assessment, such as scRNA-seq and TCR-seq., flow cytometry analysis, and tumor growth measurement. Tumor growth trajectories for individual mice across multiple treatment groups were monitored and plotted. All mice were housed under specific pathogen free conditions at Yonsei University, College of Medicine animal facility with ambient temperature 25 ± 1°C, humidity 50 ± 10%, and light cycle of 12 h. All experiments performed under the approval of the Institutional Animal Care and Use Committee at Yonsei University College of Medicine (IACUC#2022-0130).

Immunohistochemistry and quantitative image analysis

Mice were treated as previously described. Tumor tissues were dissected, and fixed in 10% formaldehyde solution over 12 h. Sectioned by 3–5 μm thick slice on coated-slide de-paraffinized and rehydrated using graded ethanol solutions. Hematoxylin stained for 3min after rinsing the slide with sterilized water, and incubated with eosin for 5min following the washes. Finally, the tissues sections were covered with coverslips with DPX mount after dehydrated with

graded ethanol. Human H&E slide were prepared with surgical samples as same described with murine slide section preparation.

Tissue preparation for single-cell dissociation

Human Tumor tissues were finely minced and processed using a gentle MACS dissociator (Miltenyi Biotec, Gladbach Bergisch, Germany, Cat#130-093-235), followed by enzymatic digestion with the Human Tumor Dissociation Kit (Miltenyi Biotec, Cat#130-095-929) according to the manufacturer's protocol. After a 1 h incubation at 37°C, the samples were filtered through a 70-µm MACS SmartStrainer (Miltenyi Biotec, Cat#130-098-462) into RPMI-1640 medium (Corning, Inc., Corning, NY, USA) supplemented with 10% fetal bovine serum (Gibco, USA), and then centrifuged at 300 ×g for 10 min. TILs were isolated using density gradient centrifugation with Ficoll (Cytiva, USA). The collected cells were washed with RPMI-1640 medium and assessed for viability and cell count using trypan blue.

Murine tissues were dissected and digested with Collagenase Type IV (Worthington, Freehold, NJ, USA), DNase I (Sigma-Aldrich, St. Louis, USA), and Penicillin-Streptomycin (Cytiva, USA) in RPMI-1640. After digestion, downstream preparation was same as human tumor dissociation protocols. Equal number of cells were pooled from each group mice.

Quantification and statistical analysis

The study was designed with null and alternative hypotheses of an MPR rate of ≤5% and ≥35%, respectively. Patient demographics, clinical characteristics, and outcomes were summarized using descriptive statistics. The Kaplan–Meier method was used to estimate the distribution of the time-to-event endpoints. In pre-clinical model, all statistical analysis was measured using Prism 8 (GraphPad Software). Data were indicated as means ± SEM or SD indicated in figure legend.

If multiple groups were compared, t-test or one-way ANOVA was performed by Tukey's post-hoc tests. And, paired Wilcoxon rank-sum tests were used for the purpose of conducting sample-wise normalized paired comparisons. In the in-silico clinical analysis, cell-wise comparisons were assessed using a two-tailed unpaired t-test, while sample-wise comparisons across multiple conditions were evaluated using a one-sample Wilcoxon signed-rank test.

For the single-cell and spatial analyses, statistics were computed panel by panel and panels were never pooled into a common multiple-testing family. Three rules were applied consistently. (1)

Panels comparing two groups, including correlation panels and panels in which each facet contains exactly two groups, are shown with nominal P values. (2) For faceted panels, Benjamini–Hochberg (BH) adjusted P values were additionally computed with the facets of that panel as the correction family. (3) Panels comparing three or more groups — comparisons of each group with the overall mean of the panel (reference ".all."), lists of pairwise comparisons, and GSEA panels showing many pathways at once — are displayed with BH-adjusted P values, with the nominal values reported alongside.

The individual tests were chosen according to the unit of analysis. Cell-level and BCR-sequence-level comparisons of two groups were assessed with the two-sided Welch t-test; sample-level comparisons across groups were assessed with the Wilcoxon rank-sum test; and paired pre- versus post-treatment comparisons were assessed with the two-sided Wilcoxon signed-rank test, with pairs matched explicitly by patient (or by patient × subset where indicated) rather than by row order. Differential expression used the two-sided Wilcoxon rank-sum test with Bonferroni correction as implemented in Seurat, and gene-set enrichment used the Wilcoxon-based gene ranking described above with BH correction across pathways. Associations between continuous variables were tested with the F-test of the corresponding linear regression, and associations involving regulon activity ratios were tested with Spearman rank correlation. The test, the correction and the unit of analysis used for every panel are stated in the corresponding figure legend.

Analytic p-values were described as shown; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. Kaplan-Meier survival analyses were performed using GraphPad 8.0 software to determine the significant difference among treatment groups.

Tables

Table 1. Baseline and treatment characteristics.

Index	Total (N=56)	Cohort I (N=11)	Cohort II (N=15)	Cohort III (N=15)	Cohort IV (N=15)
Median age (range)	58 (37-73)	52 (43-71)	58 (37-67)	63 (46-73)	53 (38-67)

Sex (patients)					
Male	50 (89.3%)	9 (81.8%)	14 (93.3%)	14 (93.3%)	13 (86.7%)
Female	6 (10.7%)	2 (18.2%)	1 (6.7%)	1 (6.7%)	2 (13.3%)
Clinical stage at baseline (patients)					
II	46 (82.1%)	8 (72.7%)	11 (73.3%)	14 (93.3%)	13 (86.7%)
III	10 (17.9%)	3 (27.3%)	4 (26.7%)	1 (6.7%)	2 (13.3%)
HPV type (patients)					
HPV 16	54 (96.4%)	11 (100.0%)	15 (100.0%)	14 (93.3%)	14 (93.3%)
HPV 18	2 (3.6%)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)
Pathologic stage at surgical resection (patients)					
0 (ypCR)	7 (12.5%)	4 (36.4%)	2 (13.3%)	1 (6.7%)	0 (0.0%)
I	31 (55.4%)	7 (63.6%)	9 (60.0%)	11 (73.3%)	4 (26.7%)
II	12 (21.4%)	0 (0.0%)	1 (6.7%)	3 (20.0%)	8 (53.3%)
III	3 (5.4%)	0 (0.0%)	1 (6.7%)	0 (0.0%)	2 (13.3%)
Surgery not performed	3 (5.4%)	0 (0.0%)	2 (13.3%)	0 (0.0%)	1 (6.7%)
Adjuvant chemoradiotherapy (patients)					

Yes	21 (39.6%)	2 (18.2%)	3 (23.1%)	7 (46.7%)	9 (64.3%)
No	32 (60.4%)	9 (81.8%)	10 (76.9%)	8 (53.3%)	5 (35.7%)

Figure legends

Fig. 1. Triple immunotherapy couple tumor control with a stemness-enriched intratumoral CD8$^+$ T-cell landscape in mice. (a) Schematic overview of treatment and pre-clinical outcome. Mice-bearing TC-1 tumor were treated anti-mouse-PD-1 250ug per 3-4 days; HPV16 E6/E7 DNA vaccine (GX188E) 0.4ug on day 0, and 7; long-acting IL-7 (GX-I7/NT-I7) 295ug on day 1. Resected tumor, and draining lymph node specimens were assessed for histology, and transcriptome analysis on each indicated time-points. (b) Kinetics of tumor volumes showing the anti-tumor effects by each treatment. (c) Overall survival during experimental duration. (d) Tumor- infiltrated CD8$^+$ lymphocytes measured by IHC analysis at 25 day-post-injection. (e) UMAP plots showing tumor-infiltrated CD8+T cells subclusters. (f) Density plot of CD8$^+$T phenotypes over pseudotime (top; pseudotime differentiation, bottom; proportional distribution). (g) Stacked bar plot showing CD8$^+$ subclusters proportion after treatments in tumor. (h) Line plot showing stemness (top), exhaustion (bottom) module scores along pseudotime, and CD8$^+$ distributed colored by treatment groups along pseudotime. Statistical analyses were performed using two-way ANOVA (b), one-way ANOVA (e), and log-rank tests (d), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. Schematics in (a) were created, and modified using BioRender.com.

Fig. 2. Clinical activity across vaccine-based neoadjuvant regimens and 2-year HPV16 E6/E7-reactive immunity after triple immunotherapy. (a) Schematic overview of the four-cohort treatment platform and clinical evaluation in resectable HPV16-positive HNSCC. Cohort I received pembrolizumab, GX-188E, and NT-I7 at 1,200 μg/kg; cohort II received pembrolizumab and GX-188E; cohort III received pembrolizumab, GX-188E, and NT-I7 at 360 μg/kg; and cohort IV received GX-188E and NT-I7 at 1,200 μg/kg. Pembrolizumab was administered on days 1 and 22, GX-188E on days 1, 8, and 22, and NT-I7 on day 8 before surgery. Resected tumors were assessed for pathologic response and translational analyses. (b)

Waterfall plot showing the pathologic response (left), bar plot showing pathologic response rate (mid), and postoperative CCRT frequency after surgery (right). (c) Representative flow cytometry plots of $CD69^+CD137^+$, and intracellular cytokines among $CD8^+$T cells. (d) Longitudinal dynamics of $CD69^+CD137^+$, and intracellular cytokines (TNF-α^+IFN-γ^+) across $CD8^+$T-cell subsets in peripheral blood samples. (e) Longitudinal dynamics of IFN-γ ELISpot in peripheral blood samples, and representative images among ELISpot analysis. (f) Representative Lunit IO SCOPE image (left), and AI-assisted quantification of immune-inflamed scores using Lunit SCOPE IO before and after treatment (right). Statistical analyses were performed using multiple comparison one-way ANOVA (d), paired t-test (f), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. Schematics in (a) were created, and modified using BioRender.com.

Fig. 3. GC-like/TLS-associated adaptive immune remodeling after neoadjuvant triple immunotherapy. (a) Schematic overview of the exploratory transcriptomic analysis of Cohort I. (b) Gene set enrichment analysis of Gene Ontology Biological Process pathways comparing post-treatment with pre-treatment tumor in all immune cells. Genes were ranked by average log2 fold-change from Seurat FindMarkers. Bars show the normalized enrichment score (NES; positive, enriched post-treatment) for immune-related pathways with nominal $P \leq 0.05$, colored by time point. (c) UMAP of B cells colored by subset, together with the monocle3 trajectory of B cells colored by branch assignment: Path1 (germinal-center-like, GC), Path2 (extrafollicular-like, EF), Mixed and None. (d) UMAP of B cells colored by the GC-related (top) and EF-related (bottom) module scores. (e) Violin and box plots of the log2 ratio of the GC to the EF module score in Path1 versus Path2 B cells. The unit of analysis is the single cell. Diamonds indicate the mean. (f) Log2 of the HPV specificity score along monocle3 pseudotime in Path1 (red) and Path2 (blue) B cells. Lines are linear regression fits with 95% confidence bands, and R^2 is shown for each branch. (g) Density of B-cell subsets along pseudotime within Path1, scaled to the total density at each pseudotime value. (h) Mean HPV specificity score per patient, tissue and B-cell subset within Path1. Each subset was compared with the overall mean (dashed line). (i) Somatic hypermutation frequency of B_AtM cells in Path1, split by class-switch status (UnSw, unswitched; Sw, switched) and compared between pre- and post-treatment tumor. The unit of analysis is the BCR sequence. (j) Representative AI-assisted spatial pathology image showing tumor-infiltrating lymphocytes (TILs) in a patient with pCR. (k) Intratumoral tertiary lymphoid structure (TLS) area normalized to cancer area before and after neoadjuvant treatment ($n = 9$). (l) Association between the post-treatment intratumoral TLS area ratio and pathological tumor regression ($n = 9$). Points are colored by response group, and the line is a linear regression fit with R^2 shown. Statistical analyses were

performed using Wilcoxon rank-sum test for gene ranking in the gene set enrichment analysis (b; nominal *P*), two-sided Welch t-test (e, i; nominal *P*), F-test of linear regression (f, l; nominal *P*), Wilcoxon rank-sum test with Benjamini–Hochberg adjustment (h; adjusted *P*), and two-sided Wilcoxon signed-rank test on matched patients (k; nominal *P*), and significance indicates *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Fig. 4. Reinvigoration and transcriptional rewiring of tumor-reactive CD8⁺T cells following neoadjuvant triple immunotherapy. (a) UMAP of CD8⁺T cells colored by subset. (b) Signature scores of CD8⁺T-cell subsets, averaged per patient, tissue and subset. Each subset was compared with the overall mean within each signature. Diamonds indicate the mean. (c) UMAP of CD8⁺T cells highlighting the five most expanded (Top5) and the next five most expanded (Top10) TCR clonotypes per response group. (d) Distribution of CD8⁺T-cell subsets across clone size categories (1, 2 and ≥ 3 cells per clonotype). (e) Volcano plot of differentially expressed genes between post- and pre-treatment tumor in tumor-reactive (TR) CD8⁺T cells. Genes with adjusted P ≤ 0.01 and |log2 fold-change| > log2(1.3) are colored, and functionally annotated genes are labeled. (f) Gene set enrichment analysis (GOBP) of the same comparison as in (e). Bars show NES for pathways with nominal P ≤ 0.05, colored by time point. (g) Signature scores of TR CD8⁺T cells before and after treatment, averaged per patient and subset (n = 9; only patient × subset pairs with both time points are shown). Gray lines connect matched pairs. Diamonds indicate the mean. (h) Heat map of selected functional genes in TR CD8⁺T-cell subsets before and after treatment. Expression is the average expression per subset and time point, z-scored per gene, and genes are grouped by functional category. (i) Associations between the post-treatment intratumoral TLS area ratio and the fraction of CD8⁺_Tex among TR CD8⁺T cells (top) and the exhaustion score of post-treatment CD8⁺_Tex cells (bottom) (n = 9). Lines are linear regression fits with R2 shown. Statistical analyses were performed using Wilcoxon rank-sum test with Benjamini–Hochberg adjustment (b; adjusted P), Wilcoxon rank-sum test with Bonferroni correction (e; adjusted P), Wilcoxon rank-sum test for gene ranking in the gene set enrichment analysis (f; nominal P), two-sided Wilcoxon signed-rank test on matched pairs (g; nominal P), and F-test of linear regression (i; nominal P), and significance indicates *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Fig. 5. Tumor–tdLN-shared *GZMK*⁺ effector-memory CD8⁺ clonotypes and treatment-associated *MYC–BATF* rebalancing after neoadjuvant immunotherapy. (a) Proportion of

TCR clonotypes assigned to each CD8$^+$T-cell subset in each tissue. (b) Post/pre ratio of the proportion of each TR CD8$^+$T-cell subset per patient (square-root scale; n = 8), shown for all patients (left) and split by response group (middle and right). Each subset was compared with the overall mean. Dashed line, no change (ratio = 1). (c) UMAP of the most expanded TCR clonotypes per CD8$^+$T-cell subset and response group in pre-treatment tumor (top; red, Top5; blue, Top10), and the number of cells belonging to these Top5 and Top10 clonotypes across tissues and response groups (bottom). (d) Volcano plot of differentially expressed genes between non-MPR and MPR/pCR patients in pre-treatment CD8$^+$*GZMK*$^+$-EM cells. Genes with adjusted $P \leq 0.01$ and |log2 fold-change| > log2(1.3) are colored. (e) Associations between the post-treatment intratumoral TLS cluster count ratio and the fraction of CD8$^+$*GZMK*$^+$-EM among TR CD8$^+$T cells (top), and between the CXCR5 module score of post-treatment CD8$^+$*GZMK*$^+$-EM cells and the post-treatment intratumoral TLS area ratio (bottom) (n = 9). Lines are linear regression fits with R2 shown. (f) Number of unique clonotypes shared between the post-treatment tumor and the post-treatment lymph node, colored by CD8$^+$T-cell subset (left), and chord diagram of the same shared clonotypes (right); ribbon width is proportional to the number of shared clonotypes connecting each subset–tissue sector. (g) Network of clonotype sharing between CD8$^+$T-cell subsets within post-treatment tumors of non-MPR patients. Node size is proportional to the number of clonotypes detected in that subset, and edge width to the number of clonotypes shared between two subsets. (h) Module scores of post-treatment CD8$^+$*GZMK*$^+$-EM cells grouped by clonotype sharing: LN only, Tumor–LN shared and Tumor only. The unit of analysis is the single cell. Diamonds indicate the mean. (i) SCENIC regulon activity in CD8$^+$*GZMK*$^+$-EM cells for the 21 transcription factors most affected by in silico deletion of IL7R (Geneformer). Bars (left axis) show the log2 ratio of the fraction of regulon-active cells in post- versus pre-treatment tumor, colored by the direction of change, and the line (right axis) shows the mean regulon AUC in CD8$^+$*GZMK*$^+$-EM cells. (j) Spearman correlations between the post/pre ratio of the median regulon AUC per patient in CD8$^+$*GZMK*$^+$-EM cells and two clinical variables, the post-treatment intratumoral TLS area ratio and pathological tumor regression (n = 9). Transcription factors with nominal $P \leq 0.05$ for at least one variable are shown. Color, Spearman R; dot size, −log10 P. (k) Regulon specificity score (RSS) of *Batf* versus *Myc* in CD8$^+$*Gzmm*$^+$-EM cells of the mouse model, z-scored within each treatment arm across all consensus regulons. Points are treatment arms of the lymph node (LN) and tumor (T), and the dashed line indicates equal z-scores. Arms with fewer than 10 cells were excluded.

Statistical analyses were performed using Wilcoxon rank-sum test (b; nominal P), Wilcoxon rank-sum test with Bonferroni correction (d; adjusted P), F-test of linear regression (e; nominal P), two-sided Welch t-test with Benjamini–Hochberg adjustment across all 12 comparisons (h; adjusted P), and Spearman rank correlation (j; nominal P), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Fig. 6. The intratumoral *MYC–BATF* program in *GZMK*$^+$ effector-memory CD8$^+$ cells is associated with HPV-reactive immunity 2 years later. (A) Heatmap showing Pearson correlations of post-treatment intratumoral *GZMK*$^+$ effector-memory regulon specificity measures with postoperative and 2-year HPV16 E6/E7-reactive AIM and IFN-γ ELISpot responses. (B) Associations between treatment-associated posttreatment-to-pretreatment changes in relative *MYC*/*BATF* and *TCF7*/*BATF* RSS measures and the 2-year peptide-responsive CD69$^+$CD137$^+$CD8$^+$ stem cell–like memory phenotype in eight evaluable patient pairs. (C) Local and distant recurrence, disease-free survival, and overall survival during long-term follow-up of cohort I after neoadjuvant triple immunotherapy. Correlations were assessed using two-tailed Pearson correlation analysis. Pearson correlation coefficients and nominal *p* values are shown.

References

1. Fan, T. *et al.* Therapeutic cancer vaccines: advancements, challenges, and prospects. *Signal Transduct Target Ther* **8**, 450 (2023).
2. Brandenburg, A., Heine, A. & Brossart, P. Next-generation cancer vaccines and emerging immunotherapy combinations. *Trends Cancer* **10**, 749-769 (2024).
3. Chi, H., Pepper, M. & Thomas, P.G. Principles and therapeutic applications of adaptive immunity. *Cell* **187**, 2052-2078 (2024).
4. Shamseddine, A.A., Burman, B., Lee, N.Y., Zamarin, D. & Riaz, N. Tumor Immunity and Immunotherapy for HPV-Related Cancers. *Cancer Discov* **11**, 1896-1912 (2021).
5. Ramos da Silva, J. *et al.* Single immunizations of self-amplifying or non-replicating mRNA-LNP vaccines control HPV-associated tumors in mice. *Sci Transl Med* **15**, eabn3464 (2023).
6. Massarelli, E. *et al.* Combining Immune Checkpoint Blockade and Tumor-Specific Vaccine for Patients With Incurable Human Papillomavirus 16-Related Cancer: A Phase 2 Clinical Trial. *JAMA Oncol* **5**, 67-73 (2019).
7. Wieland, A. *et al.* Defining HPV-specific B cell responses in patients with head and neck cancer. *Nature* **597**, 274-278 (2021).
8. Eberhardt, C.S. *et al.* Functional HPV-specific PD-1(+) stem-like CD8 T cells in head and neck cancer. *Nature* **597**, 279-284 (2021).
9. Sung, H. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **71**, 209-249 (2021).

10. Chow, L.Q.M. Head and Neck Cancer. *N Engl J Med* **382**, 60-72 (2020).
11. Machtay, M. *et al.* Factors associated with severe late toxicity after concurrent chemoradiation for locally advanced head and neck cancer: an RTOG analysis. *J Clin Oncol* **26**, 3582-3589 (2008).
12. Holsinger, F.C. *et al.* Transoral Robotic Surgery in the Multidisciplinary Care of Patients With Oropharyngeal Squamous Cell Carcinoma: ASCO Guideline. *J Clin Oncol* **43**, 1369-1392 (2025).
13. Mody, M.D., Rocco, J.W., Yom, S.S., Haddad, R.I. & Saba, N.F. Head and neck cancer. *Lancet* **398**, 2289-2299 (2021).
14. Ferris, R.L. *et al.* Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. *N Engl J Med* **375**, 1856-1867 (2016).
15. Burtness, B. *et al.* Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* **394**, 1915-1928 (2019).
16. Uppaluri, R. *et al.* Neoadjuvant and Adjuvant Pembrolizumab in Locally Advanced Head and Neck Cancer. *N Engl J Med* **393**, 37-50 (2025).
17. Topalian, S.L. *et al.* Neoadjuvant immune checkpoint blockade: A window of opportunity to advance cancer immunotherapy. *Cancer Cell* **41**, 1551-1566 (2023).
18. Topalian, S.L., Taube, J.M. & Pardoll, D.M. Neoadjuvant checkpoint blockade for cancer immunotherapy. *Science* **367** (2020).
19. Kim, T.J. *et al.* Clearance of persistent HPV infection and cervical lesion by therapeutic DNA vaccine in CIN3 patients. *Nat Commun* **5**, 5317 (2014).
20. Youn, J.W. *et al.* Pembrolizumab plus GX-188E therapeutic DNA vaccine in patients with HPV-16-positive or HPV-18-positive advanced cervical cancer: interim results of a single-arm, phase 2 trial. *Lancet Oncol* **21**, 1653-1660 (2020).
21. Lee, K.J. *et al.* IL-7-primed bystander CD8 tumor-infiltrating lymphocytes optimize the antitumor efficacy of T cell engager immunotherapy. *Cell Rep Med* **5**, 101567 (2024).
22. Kim, S. *et al.* A single administration of hIL-7-hyFc induces long-lasting T-cell expansion with maintained effector functions. *Blood Adv* **6**, 6093-6107 (2022).
23. Beltra, J.C. *et al.* Stat5 opposes the transcription factor Tox and rewires exhausted CD8(+) T cells toward durable effector-like states during chronic antigen exposure. *Immunity* **56**, 2699-2718 e2611 (2023).
24. Ferris, R.L. *et al.* Neoadjuvant nivolumab for patients with resectable HPV-positive and HPV-negative squamous cell carcinomas of the head and neck in the CheckMate 358 trial. *J Immunother Cancer* **9** (2021).
25. Kim, C.G. *et al.* A Phase II Open-Label Randomized Clinical Trial of Preoperative Durvalumab or Durvalumab plus Tremelimumab in Resectable Head and Neck Squamous Cell Carcinoma. *Clin Cancer Res* **30**, 2097-2110 (2024).
26. Lim, M.C. *et al.* GX-188E DNA vaccine plus pembrolizumab in HPV 16- and/or 18-positive recurrent or advance cervical cancer: a phase 2 trial. *EClinicalMedicine* **74**, 102716 (2024).
27. Sievers, C.M. *et al.* GZMK expression within activated intratumoral T-cell subsets reflects differentiation efficiency and predicts response to cancer immunotherapy. *NPJ Precis Oncol* **10** (2026).
28. Li, H. *et al.* Distinct CD8(+) T cell dynamics associate with response to neoadjuvant cancer immunotherapies. *Cancer Cell* **43**, 757-775 e758 (2025).

29. Duquette, D. *et al.* Human Granzyme K Is a Feature of Innate T Cells in Blood, Tissues, and Tumors, Responding to Cytokines Rather than TCR Stimulation. *J Immunol* **211**, 633-647 (2023).
30. Jonsson, A.H. *et al.* Granzyme K(+) CD8 T cells form a core population in inflamed human tissue. *Sci Transl Med* **14**, eabo0686 (2022).
31. Li, H. *et al.* Mature tertiary lymphoid structures evoke intra-tumoral T and B cell responses via progenitor exhausted CD4(+) T cells in head and neck cancer. *Nat Commun* **16**, 4228 (2025).
32. Afeyan, A.B. *et al.* Tertiary lymphoid structures harbour stem-like tumour-specific T cells. *Nature* (2026).
33. Shapiro, D.D. *et al.* CXCL13-CXCR5 signaling in CD8(+) T cell recruitment and lymphoid immune organization in clear cell renal cell carcinoma. *Cancer Immunol Immunother* (2026).
34. Rahim, M.K. *et al.* Dynamic CD8(+) T cell responses to cancer immunotherapy in human regional lymph nodes are disrupted in metastatic lymph nodes. *Cell* **186**, 1127-1143 e1118 (2023).
35. Turner, C.T. Pro-inflammatory granzyme K contributes extracellularly to disease. *Front Immunol* **16**, 1620670 (2025).
36. Wijesinghe, S.K.M. *et al.* Lymph-node-derived stem-like but not tumor-tissue-resident CD8(+) T cells fuel anticancer immunity. *Nat Immunol* **26**, 1367-1383 (2025).
37. Baharom, F. *et al.* Intravenous nanoparticle vaccination generates stem-like TCF1(+) neoantigen-specific CD8(+) T cells. *Nat Immunol* **22**, 41-52 (2021).
38. Kim, G.M. *et al.* GX-I7, a long-acting IL-7, safely and effectively increased peripheral CD8(+)/CD4(+) T cells and TILs in patients with locally advanced or metastatic solid tumours. *Br J Cancer* **133**, 524-532 (2025).
39. Jang, H. *et al.* Long-acting IL-7 induces distinct transcriptomic features in peripheral T cells of patients with solid tumors. *JCI Insight* **11** (2026).
40. Park, H.J. *et al.* Transcriptomic analysis of human IL-7 receptor alpha (low) and (high) effector memory CD8(+) T cells reveals an age-associated signature linked to influenza vaccine response in older adults. *Aging Cell* **18**, e12960 (2019).
41. Frumento, G. *et al.* Homeostatic Cytokines Drive Epigenetic Reprogramming of Activated T Cells into a "Naive-Memory" Phenotype. *iScience* **23**, 100989 (2020).
42. Frey, A. *et al.* Targeting an essential viral oncoprotein with an IL-7-enhanced mRNA vaccine induces durable immunity to Merkel cell carcinoma. *Cell Rep* **44**, 116359 (2025).
43. Chen, M. *et al.* Long-Acting Recombinant IL-7 (rhIL-7-hyFc) Enhances the Primary and Memory Neoantigen-Specific Immune Response to Breast Cancer Personalized Cancer Vaccines. *Cancers (Basel)* **17** (2025).
44. Seo, H. *et al.* BATF and IRF4 cooperate to counter exhaustion in tumor-infiltrating CAR T cells. *Nat Immunol* **22**, 983-995 (2021).
45. Zhang, X. *et al.* Depletion of BATF in CAR-T cells enhances antitumor activity by inducing resistance against exhaustion and formation of central memory cells. *Cancer Cell* **40**, 1407-1422 e1407 (2022).
46. Luoma, A.M. *et al.* Tissue-resident memory and circulating T cells are early responders to pre-surgical cancer immunotherapy. *Cell* **185**, 2918-2935 e2929 (2022).
47. Sethna, Z. *et al.* RNA neoantigen vaccines prime long-lived CD8(+) T cells in pancreatic cancer. *Nature* **639**, 1042-1051 (2025).

48. Aggarwal, C. *et al.* Immunotherapy Targeting HPV16/18 Generates Potent Immune Responses in HPV-Associated Head and Neck Cancer. *Clin Cancer Res* **25**, 110-124 (2019).
49. Komdeur, F.L. *et al.* CD103+ tumor-infiltrating lymphocytes are tumor-reactive intraepithelial CD8+ T cells associated with prognostic benefit and therapy response in cervical cancer. *Oncoimmunology* **6**, e1338230 (2017).

Acknowledgments

The authors specially thank Genexine Inc. and NeoImmuneTech Inc. for providing the GX-188E (tirvalimogene teraplasmid), and GX-I7/NT-I7 (efineptakin alfa).

Funding

This study was supported by the National Research Foundation funded by the Ministry of Science and ICT grant RS-2022-CC125144 (HRK); National Research Foundation funded by the Korea government grant RS-2026-25481453 (HRK); National Research Foundation funded by the Ministry of Science and ICT grant RS-2025-02214844 (HRK); National Research Foundation funded by the Korea government RS-2025-18362970 (HRK); Yonsei University College of Medicine grant 6-2022-0141 (HRK); Yonsei Fellow Program funded by Youn Jae Lee (HRK),

National Research Foundation funded by the Ministry of Science and ICT grant RS-2023-00261820 (MHH),

National Research Foundation funded by the Ministry of Science and ICT grant RS-2024-00348654 (CGK); Korea Health Technology R&D Project through the Korea Health Industry Development Institute funded by the Ministry of Health and Welfare grant RS-2024-00405569 (CGK); Yonsei University College of Medicine grant 4-2023-1187 (CGK); Yonsei University College of Medicine grant 6-2023-0136 (CGK); Yonsei University College of Medicine grant 6-2026-0055 (CGK); Yonsei University College of Medicine grant 6-2026-0089 (CGK),

National Research Foundation funded by the Bio & Medical Technology Development Program grant RS-2023-00222762 (JYK); Korea Health Technology R&D Project through the Korea Health Industry Development Institute funded by the Ministry of Health and Welfare grant RS-2024-00440385 (JYK)

Brain Korea 21 (BK21) FOUR program

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Competing interests

CGK reported consulting or advisory role from Pfizer, Amgen, Novartis, Johnson & Johnson/Janssen, and Ono Pharmaceutical, speakers' bureau from Astellas, Takeda, Boehringer Ingelheim, Novartis, Merck, AstraZeneca, MSD Oncology, Hanmi, and Dong-A ST, and research funding from Ipsen. HRK reported consulting or advisory role from Bayer, AstraZeneca, Bristol Myers Squibb, Takeda, and Yuhan, speakers' bureau from AstraZeneca, Bristol Myers Squibb, and Genentech/Roche, research funding from Yuhan, Takeda, AstraZeneca, AFFIMED, and Maia Biotechnology. Others have declared no conflicts of interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability

All customized scripts to process in this study are available upon request. Any additional information required to re-analyze the data represented in this paper is available from lead contact (nobelg@yuhs.ac) upon reasonable request.

Extended data

Extended Data Fig. 1 Tumor growth kinetics and histologic examination of $CD8^+$T cell infiltration in murine model, related to Figure 1.

(A) Individual tumor growth curves by each group. (B) Waterfall plot showing tumor regression rates (%) (n=15-20). (C) Representative immunohistochemistry images (10x) illustrating $CD8^+$ infiltration, and representative hematoxylin and eosin (H&E) images (10x) of each group.

Extended Data Fig. 2 Murine $CD8^+$T cell subclusters and functional profiling, related to Figure 1.

(A) UMAP visualization of whole immune cell subsets. (B) Dot plots showing marker gene expression across whole immune cell subsets. (C) Boxplots showing $CD8^+$T signature scores of stemness, effector, exhaustion, and proliferation (D) Dot plots showing transcriptional marker expression in whole $CD8^+$T.

Extended Data Fig. 3 Radiologic response, related to Figure 2

(A) Waterfall plot showing the radiologic response rates based on tumor volume reduction (left), and bar plot showing radiologic response rate (right).

Extended Data Fig. 4 Spatial analysis of tumor-infiltrating lymphocytes, related to Figure 2.

AI-assisted quantification of lymphocytes infiltration density using Lunit SCOPE IO before and after treatment. Density quantification of intratumoral (left) and stromal (right) TIL quantified using Lunit SCOPE IO. Statistical testing performed along matched samples according to a two-tailed paired t-test. Data are presented as mean ± s.d. TIL; tumor-infiltrating lymphocyte. Significance is indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Extended Data Fig. 5 Longitudinal analysis demonstrates durable induction of HPV16 vaccine-reactive T cells in all patients, related to Figure 2.

(A) Representative flow cytometry plots for AIM assay (left), longitudinal dynamics of $CD69^+CD137^+$across $CD8^+$T-cell subsets in peripheral blood samples (right). (B) Bar plot showing longitudinal dynamics of IFN-γ ESLIPOT, and differences in IFN-γ ELISPOT

responses. (C) Representative IFN-γ ESLIPOT images in whole patients. Statistical analyses were performed using paired t-test (A), multiple comparison one-way ANOVA (B), and significance indicates *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Extended Data Fig. 6 Spatial distribution of tumor-infiltrating lymphocytes, related to Figure 4.

(A) Representative AI-assisted images (20×) illustrating TIL distribution in tumor tissues for all patients.

Extended Data Fig. 7 Annotation and quantitative changes of major cell types, related to Figure 3.

(a) UMAP of all cells colored by major lineage. (b) Dot plot of canonical marker genes per major lineage. Dot size, fraction of expressing cells; color, scaled mean expression. (c) UMAP feature plots of the marker genes shown in (b). (d) Post/pre ratio of the proportion of each immune lineage per patient (square-root scale; n = 8), shown for all patients (left) and split by response group (middle and right). Each lineage was compared with the overall mean. Statistical analyses were performed using Wilcoxon rank-sum test (d; nominal P), and significance indicates **p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.*

Extended Data Fig. 8 Quality control and further analysis of B cell subsets, related to Figure 3.

(a) UMAP feature plots of B-cell marker genes. (b) Dot plot of the same genes per B-cell subset. Dot size, fraction of expressing cells; color, scaled mean expression.

Extended Data Fig. 9 B cell sub-analysis supplementary, related to Figure 3.

(a) Log2 of the HPV specificity score in Path1 versus Path2 B cells. The unit of analysis is the single cell. Diamonds indicate the mean. (b) Associations between the mean GC score and the mean HPV specificity score (left) and the mean somatic hypermutation frequency (right), computed per patient and B-cell subset within Path1. Lines are linear regression fits with R2 shown. (c) GC/EF module score ratio of B_AtM cells in Path1 before and after treatment, split by response group. Diamonds indicate the mean. (d) Density of B-cell subsets along pseudotime within Path2. (e) Mean GC score per patient, tissue and B-cell subset within Path1. Each subset

was compared with the overall mean. (f) BCR clonal diversity (inverse Simpson index, square-root scale) per patient, class-switch status, tissue and B-cell subset within Path1. Each subset was compared with the overall mean (dashed line) as in (e). (g) GC module score of B_AtM cells in Path1 before and after treatment. The unit of analysis is the single cell. Diamonds indicate the mean. Statistical analyses were performed using two-sided Welch t-test (a, c, g; nominal P), F-test of linear regression (b; nominal P), and Wilcoxon rank-sum test with Benjamini–Hochberg adjustment (e, f; adjusted P), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Extended Data Fig. 10 Spatial analysis of tertiary lymphoid structures in tumors, related to Figure 3.

(A) Representative AI-assisted spatial-pathology images illustrating tertiary lymphoid structures in tumor tissues across all patients.

Extended Data Fig. 11 Quality control and further analysis of NK/CD8$^+$T cells, related to Figure 4.

(a) UMAP feature plots of T/NK marker genes. (b) UMAP of T/NK cells colored by subset. (c) Dot plot of marker genes per CD8$^+$T-cell subset. (d) Signature scores of each TR CD8$^+$T-cell subset before and after treatment, averaged per patient (n = 9; only patient × subset pairs with both time points are shown). Gray lines connect matched pairs. Diamonds indicate the mean. (e) Difference between the z-scored IFN-γ signature and the ISG resistance signature in TR CD8$^+$T cells, averaged per patient, tissue and subset. Each subset was compared with the overall mean (left), and pre- and post-treatment tumor were compared within each subset (right). Diamonds indicate the mean. Statistical analyses were performed using two-sided Wilcoxon signed-rank test on matched pairs (d; nominal P), Wilcoxon rank-sum test with Benjamini–Hochberg adjustment (e, left; adjusted P), and Wilcoxon rank-sum test (e, right; nominal P), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Extended Data Fig. 12 Additional characterization of GZMK$^+$ CD8$^+$T cells, related to Figure 5

(a) Gene set enrichment analysis (GOBP) of CD8$^+$*GZMK*$^+$-EM cells versus all other CD8$^+$T cells in post-treatment tumors. Bars show NES for pathways with nominal $p \leq 0.01$. (b) TCR

repertoire similarity (Tversky index) between CD8$^+$T-cell subsets of the post-treatment tumor and the post-treatment lymph node of the same patient. Each subset pair was compared with the overall mean (dashed line). (c) SCENIC regulon activity in CD8$^+$*GZMK*$^+$-EM cells for the 50 transcription factors most affected by in silico deletion of IL7R (Geneformer). Bars (left axis) show the log2 ratio of the fraction of regulon-active cells in post- versus pre-treatment tumor, and the line (right axis) shows the mean regulon AUC in CD8$^+$*GZMK*$^+$-EM cells. (d) Association between the post/pre ratio of the median regulon AUC per patient in CD8$^+$*GZMK*$^+$-EM cells and pathological tumor regression, shown for *BATF* and *MYC* (n = 9). Points are colored by response group, and the dashed line is a linear regression fit. Spearman R is shown for each transcription factor. (e) Association between the same post/pre regulon AUC ratios as in (d) and the post-treatment intratumoral TLS area ratio (n = 9). (f) Genes whose embeddings were most affected by i*n silico* deletion of IL7R in CD8$^+$*GZMK*$^+$-EM cells (Geneformer). The mean cosine distance between the perturbed and unperturbed gene embeddings is plotted against the number of cells in which the gene was detected, restricted to genes in the top 40% by detection and in the lowest 40% by cosine similarity. Genes listed in Fig.5J are labeled and key transcription factors are colored red. (g) The same analysis as in (f) performed on CD8$^+$*Gzmm*$^+$-EM cells of the mouse model. Statistical analyses were performed using Wilcoxon rank-sum test for gene ranking in the gene set enrichment analysis (a; nominal P), Wilcoxon rank-sum test with Benjamini–Hochberg adjustment (b; adjusted P), and Spearman rank correlation (d, e; nominal P), and significance indicates *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Extended Data Fig. 13 Murine CD8$^+$Gzmm$^+$-EM T cell subclusters and functional profiling, related to Figure 5.

(a) UMAP plots showing CD8$^+$ singleton or expanded clonotype (left) in tumor CD8$^+$T cells, and visualization of tumor-reactive, and bystander CD8$^+$T (right). (b) Box plots showing expression scores for stemness/memory, T-activation, Effector transcription marker across CD8$^+$T cell subsets per sample. Statistical testing by unpaired Wilcoxon rank-sum test. (c) Bar plot showing tumor-lymph node overlapped TCR proportion in whole CD8$^+$T cells (top), Among overlapped TCR (colored in top), alluvial plots showing the overlapped diversity between lymph nodes and tumor in each group (bottom). (d) Network plot showing overlapping clonotypes in tumors. Node size indicates clone size; edge width indicates overlap strength between subsets. (e) Pseudotime analysis (Slingshot) showing CD8$^+$T cell differentiation lineages, colored by cell

type. (f) Barplot showing the log2 *Myc*/*Batf* mean AUC in whole murine CD8$^+$T cells calculated with SCENIC. (g) Barplot showing the log2 *Myc*/*Batf* mean AUC in murine CD8$^+$*Gzmm*$^+$-EM cells calculated with SCENIC. (h) Dotplot showing stemness or differentiation mediated transcription gene expression in murine CD8$^+$*Gzmm*$^+$-EM. (i) Boxplots showing murine CD8$^+$*Gzmm*$^+$-EM gene set correlation score calculated by human CD8$^+$*Gzmm*$^+$-EM. Dahsed line indicates mean of whole gene set signature scores. (j) GO analysis showing the characteristics of murine CD8$^+$*Gzmm*$^+$-EM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$, according to a Wilcoxon rank paired group test.

Extended Data Fig. 14 Phenotype of vaccine-induced peripheral memory CD8$^+$ T cells and correlations with intratumoral GZMK$^+$ effector-memory CD8$^+$ transcriptional programs. (a) Representative longitudinal memory CD8$^+$T cell FACS plot (left), and proportion of memory CD8+T cell subsets in longitudinal blood samples collected from all patients (right). (b) Representative longitudinal memory CD8$^+$TSCM, and Ag$^+$TSCM FACS plot (left), and proportion of stem-like memory characteristics in longitudinal blood samples collected from all patients (right). TEMRA, terminally differentiated effector T cells; TEM, effector memory T cells; TCM, central memory T cells; Naive, Naive T cells; TSCM, stem-like memory phenotype. (c-d) Correlation between postoperative or 2-year peripheral CD69$^+$CD137$^+$ responses and SCENIC RSS of intratumoral CD8$^+$*GZMK*$^+$-EM T cells. (e) Correlation between 2-year peripheral CD8+TSCM and SCENIC RSS of intratumoral CD8$^+$*GZMK*$^+$-EM T cells. Correlations were assessed using two-tailed Pearson's correlation analysis. Pearson's correlation coefficient (r) and p values are indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$.

Extended Table 1 Adverse events

Supplementary Files

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