Published in final edited form as:

Laryngoscope. 2017 December ; 127(12): 2777–2783. doi:10.1002/lary.26679.

Barriers to Generating PDX Models of HPV-Related Head and Neck Cancer

Nicole D Facompre, PhD, Varun Sahu, BS, Kathleen T. Montone, MD, Kayla M. Harmeyer, PhD, Hiroshi Nakagawa, MD PhD, Anil K. Rustgi, MD, Gregory S. Weinstein, MD, Phyllis A. Gimotty, PhD, and Devraj Basu, MD PhD

Department of Otorhinolaryngology-Head and Neck Surgery (N.D.F., V.S., K.M.H., G.S.W., D.B.), Department of Pathology (K.T.M.), Department of Medicine (H.N., A.K.R.), Department of Biostatistics and Epidemiology (P.A.G.), The University of Pennsylvania, the Philadelphia VA Medical Center (D.B.), and the Wistar Institute (D.B.), Philadelphia, Pennsylvania, U.S.A.

Abstract

Objectives/Hypothesis—Delineate factors impacting creation and use of patient-derived xenografts (PDXs) of human papilloma virus-related (HPV+) head and neck squamous cell carcinomas (HNSCCs).

Study Design—Laboratory-based translational study.

Methods—51 surgically resected HNSCCs, including 31 HPV+ cancers, were implanted into NOD/SCID/IL-2R $\gamma^{-/-}$ (NSG) mice using standardized methodology. Clinical and pathologic factors were tested for association with engraftment. The gross, histologic, and molecular features of established HPV+ PDXs were analyzed in comparison to their tumors of origin.

Results—Negative HPV status and perineural invasion (PNI) were independent, additive factors associated with increased PDX formation. Epstein-Barr Virus-positive (EBV+) human large B-cell lymphomas grew from 32% of HPV+ HNSCC cases that failed to engraft. Successfully established HPV+ PDXs retained basaloid histology and often developed cystic growth patterns typical of HPV+ nodal metastases. They also maintained elevated p16^{INK4A} levels and expression of E6/E7 viral oncogene transcripts.

Conclusion—Reduced engraftment by HPV+ tumors lacking PNI likely results in selection biases in HNSCC PDX models. Formation of EBV+ lymphomas in NSG mice further reduces generation of HPV+ models and must be ruled out before long-term use of PDXs. Nevertheless, retention of distinctive pathologic traits and viral oncogene expression by HPV+ PDXs provides a viable *in vivo* platform for basic and translational studies as well as a resource for generating advanced *in vitro* models.

Send correspondence to Devraj Basu MD PhD, Department of Otorhinolaryngology-Head and Neck Surgery, 31400 Spruce St., 5 Ravdin/ Silverstein, Philadelphia, PA 19104. devraj.basu@uphs.upenn.edu. Telephone: 215-662-2777.

Triological Society Thesis 2017-5

Disclosures: The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Keywords

Patient derived xenograft; HPV; head and neck cancer; preclinical model

Introduction

Head and neck cancer epidemiology has been altered by declining smoking and shifting sexual practices in the US along with recognition of HPV+ HNSCC as a distinct clinical entity^{1, 2}. Decreases in smoking-related cases are accompanied by the ongoing rise in incidence of HPV+ tumors³. These viral oncogene-driven cancers preferentially arise in the lymphoid-rich tissues of the oropharynx over other sites⁴ and occur in younger, predominantly male patients with lower tobacco exposure². Distinctive traits of HPV+ oral cancer include small or occult primary tumors arising in tonsillar crypts, high rates of lymph node metastasis, cystic nodal architecture, and basaloid differentiation. HPV+ disease is also a molecularly distinct subgroup, where viral oncogene expression is associated with fewer p53 mutations, more PI3-kinase activating alterations, and a less complex mutational landscape^{5–7}. HPV+ tumors also usually overexpress the p16^{INK4A} tumor suppressor⁸, a sequela of HPV viral oncogene function that is otherwise uncommon in malignancy.

Aggressive multimodality therapies standardly used for advanced HNSCCs have higher efficacy in the HPV+ subgroup⁹. The resulting high cure rates in a younger population create long-term survivors with lifelong treatment-related morbidities from combining radiation with cytotoxic chemotherapy. These problems justify current efforts to de-escalate radiation and chemotherapy in order to mitigate their lasting toxicities without compromising oncologic outcomes. Clinical trials with this objective have largely sought to limit or alter delivery of the existing standard treatments¹⁰ rather than define novel therapies. Although HPV+ cancer-specific targeted therapies might abate toxicity and improve efficacy, their development is impaired by limited understanding of the molecular mechanisms and vulnerabilities of this disease.

The paucity of models for HPV+ HNSCC is a barrier to basic understanding and preclinical evaluation of therapeutics. Under-representation of HPV+ cancer in basic studies is at least partly related to the weak *in vitro* growth potential of HPV+ patient-derived specimens, which has resulted in few available HPV+ HNSCC cell lines¹¹. Thus most basic research is still performed using HPV- models despite HNSCC incidence shifting toward the HPV+ demographic. The rarity of HPV+ HNSCCs that establish stable cell lines may also produce a selection bias against the molecular traits of more typical HPV+ cancers in the few HPV+ lines that exist.

The high efficiency of human tumor engraftment in immunodeficient mice holds potential to address the scarcity of HPV+ HNSCC models and selection biases inherent in existing ones. Such patient-derived xenografts (PDXs) were first described in 1969¹² but only recently found widespread application. PDXs have limited mutational drift relative to cancer cell lines^{13, 14} and better retain genetic and epigenetic features of their tumors of origin^{15, 16}, including intra-tumor heterogeneity. Large PDX collections spanning multiple malignancies have shown potential as high throughput drug screening tools¹⁷. PDXs are also finding

clinical application for personalizing therapy based on the concept of "co-clinical trials" that screen second-line drugs using a patient's own engrafted malignancy¹⁶. Recognition of PDXs as essential modern cancer research tools has also led to formation of consortia to create, maintain, and disseminate large collections with clinical and molecular annotation¹⁷.

PDXs of HPV+ HNSCCs are previously described^{15, 18–21} but have yet to be widely adopted for basic and translational studies. This limitation might result from lower rates of primary surgery for HPV+ cancer producing fewer specimens for research use. In addition, these less aggressive cancers may have unfavorable growth properties as xenografts that prevent stable PDX maintenance during serial *in vivo* passage. Nevertheless, the stable engraftment efficiency of HPV+ versus HPV- HNSCCs in immunodeficient mice remains to be defined using standardized methodology. Here we hypothesized that HPV+ HNSCCs have sufficient engraftment potential for their PDXs to achieve standard research utility; we thus aimed to establish a well-characterized set of HPV+ PDXs for long-term use. We further evaluated whether PDXs maintain the distinct clinical and molecular features of HPV+ HNSCCs. These questions were addressed by generating PDXs from a surgical practice performing a high volume of primary resections of HPV+ HNSCCs. In the process, emphasis was placed on standardizing methodology, ensuring stable *in vivo* passage, and directly confirming HPV viral oncogene expression. This study included both HPV+ and HPV- tumors but gave priority to untreated HPV+ primary tumors of the oropharynx resected by trans-oral surgery, which were collected without bias after ensuring adequate tissue for research use. This approach allowed characterization of the largest series of stable HPV+ PDXs reported to date.

Materials and Methods

Patients

Tissue was collected from HNSCC patients undergoing primary surgical resections at the Hospital of the University of Pennsylvania (protocol #417200) or the Philadelphia VA Medical Center (protocol #01090). Selection criteria were a T2 primary tumor (n=51) or bulky lymph node metastasis (n=4) based on preoperative imaging, as well as excess tissue available for research without compromising margin analysis. Specimens were collected over one year from the practices of two surgeons. Collection was prioritized for oropharynx cancers determined to be p16^{INK4A} positive on biopsy pathology, which were all removed by trans-oral robotic or laser resection (n=31).

PDX establishment

NSG mice were used under Wistar Institute IACUC protocol #112652. Finely minced specimens were stored in HBSS on ice and implanted within 1 hour of resection in a subcutaneous pocket in the flank. They were passaged by the same procedure after reaching ~1cm³.

Real time quantitative polymerase chain reaction (RT-qPCR)

RNA was isolated and cDNA synthesized using Qiagen RNeasy (Qiagen, Valencia, PA) and High-Capacity RNA-to-cDNA (Applied Biosystems, Foster City, CA), respectively. qPCR was performed using Power SYBR-Green Master Mix (Applied Biosystems). Primers were:

E6-Forward:5'-TCAGGACCCACAGGAGCG-3'

E6-Reverse:5'-CCTCACGTGCGCAGTAACTGTTG-3'

E7-Forward:5'-CCGGACAGAGCCCCATTACAA-3'

E7-Reverse:5'-CGAATGTCTACGTGTGTGCTTTG-3'

p16-Forward:5'-AGCATGGAGCCTTCGGCTGA-3'

p16-Reverse:5'-CCATCATCATGACCTGGATCG-3'

Immunohistochemistry (IHC) and In Situ Hybridization (ISH)

Mouse monoclonal anti-p16^{INK4A} (BD, San Jose, CA) and rabbit polyclonal anti-pan-cytokeratin (Invitrogen, Carlsbad, CA) were used to stain 5µm FFPE sections by IHC, as previously described²². Mouse monoclonal anti-CD3 (Dako, Carpinteria, CA) and anti-CD20 (Leica, Buffalo Grove, IL) were used for IHC by the Leica Bond-IIITM and Bond Polymer Refine Detection System, per manufacturer protocol. A probe for Epstein-Barr Virus (EBV)-encoded RNA 1 (EBER1) transcripts (Leica) was used for ISH using the same detection system.

Statistics

Engraftment rates of primary HNSCCs were compared among subgroups by Fisher's exact test, with exclusion of lymph node-derived PDXs from the analysis. Factors with different engraftment rates ($p < 0.05$) were analyzed using a multivariable logistic regression. Survival was estimated from Kaplan-Meier curves. Error bars represent standard errors of the means.

Results

Clinical features of the study cohort

To facilitate comparison of HPV+ versus HPV- HNSCC engraftment efficiency, a standardized protocol for generating PDXs in NSG mice was applied over a 12-month period (Fig 1A). HNSCCs growing after initial subcutaneous implantation (P0) were confirmed to sustain growth for at least one *in vivo* passage (P1) before being live-cryopreserved for long-term use. The protocol was applied to 51 specimens, of which 6 were lost from host mice acquiring *Corynebacterium bovis* infection. In the final series ($n=45$), 29 (64%) were HPV+ oropharynx cancers, defined by IHC for p16^{INK4A}. This cohort showed the expected clinical behavior in the HPV+ subgroup based on its significantly lower T-stage despite comparable overall stage (Fig 1B). Although the median follow up was only 12 and 9 months for the respective HPV- and HPV+ subsets, 100% survival of HPV+ patients at 12 months contrasted with 58% 12-month disease-specific survival of HPV- patients (Fig. 1C). Overall, these results demonstrated the cohort to have typical clinical features of advanced

stage HPV+ versus HPV- subtypes and thus facilitated comparative analysis of PDX engraftment efficiency.

Negative HPV status and perineural invasion predict PDX formation

PDXs were established from 36% of the cohort, a lower rate than typically reported for HNSCCs^{18, 19, 21, 23}. A factor underlying low engraftment in this series was an intentional bias toward collecting HPV+ tumors, which were 64% of cases but engrafted at a rate of 24%, in contrast to 56% engraftment of HPV- cases. Clinical and pathologic correlates of engraftment were determined for the 42 cases where a primary tumor was implanted. The three other specimens were from lymph nodes (2 HPV+, 1 HPV-) with bulky metastases and extracapsular extension; successful growth of all three suggested distinct engraftment behavior and warranted their exclusion here. In univariate analysis, engraftment was analyzed for association with T-stage, overall stage, smoking, perineural invasion (PNI), lymphovascular invasion, extracapsular extension, and HPV status (Table 1). Only HPV-status and PNI demonstrated significant positive association with PDX formation. By multivariate analysis, these two factors had significant additive contribution to engraftment, with higher rates when PNI was present for either HPV+ or HPV- tumors (Fig. 2). Adjusted odds ratios for PNI and HPV status were 5.2 (95% confidence interval, 1.1–25.5) and 0.21 (95% confidence interval, 0.05–0.93), respectively. In sum, these results reveal that primary HPV+ HNSCCs form PDXs with lower efficiency while also showing positive association of PNI with engraftment, independent of HPV status.

EBV+ lymphoma formation diminished PDX yields from HPV+ tumors

HNSCCs failing engraftment (n=29) included eight tumors (28%) growing at the implant site that were non-epithelial in origin based on cytokeratin staining (Fig. 3, left), with seven such instances (88%) arising from the HPV+ group. All eight tumors contained a polymorphous population of atypical lymphoid cells with plasmacytic differentiation and enlarged “Reed-Sternberg” cells. Presence of diffuse membrane CD20 allowed categorization of all cases as human diffuse B-cell lymphomas, which also retained scattered reactive human T-cells based on CD3 staining (Fig. 3, middle). ISH for EBER1 showed that all expressed EBV-derived transcripts (Fig. 3, right), suggesting their outgrowth was related to loss of immune surveillance for latent EBV infection after implantation in NSG mice. Lymphoma formation by 24% of implanted HPV+ HNSCCs suggested a sizeable negative impact of this phenomenon on HPV+ PDX generation. By contrast, lymphomas formed from 6% of HPV- tumors, a difference that did not reach statistical significance (p=0.22).

PDXs retain gross, histologic, and molecular traits of HPV+ HNSCC

All HPV+ PDXs maintained a moderately or poorly differentiated basaloid appearance with scant keratinization, leading to use of pan-cytokeratin staining to confirm their epithelial origin (not shown). The frequent cystic character of HPV+ nodal metastases was represented in multiple HPV+ PDXs, whose gross features ranged from solitary cysts to multilocular aggregates replacing most of the mass (Fig. 4A, top). This gross architecture derived histologically from cyst walls containing tumor (Fig. 4A bottom) and appeared in 4/7 (57%) HPV+ PDXs but only 1/9 (11%) HPV- PDXs, a difference with marginal significance (p=0.10). Molecular markers of HPV+ cancer were also maintained by all 7 PDXs,

including intense uniform upregulation of the p16^{INK4A} host surrogate marker used originally to classify the tumors of origin (not shown). Given imperfect specificity of p16^{INK4A} upregulation for HPV viral oncogenesis, presence of E6 and E7 viral oncogene transcripts from the prevalent HPV-16 subtype was tested by qRT-PCR. Both transcripts were detectable in 7/7 PDXs in the presumptive HPV+ group, with PDXs in the HPV- group defining a uniform non-specific background (Fig. 4B). Together these results demonstrated the retention of gross, pathologic, and molecular traits of PDXs derived from HPV-associated cancers. They further confirmed correct assignment of PDXs to HPV+ versus HPV- groups based the clinical classification of their tumors of origin.

Discussion

To date, the availability of HPV+ HNSCC PDXs for research has been low, suggesting barriers to their use despite multiple publications documenting their successful engraftment^{15, 18–21}. Furthermore engraftment rates are not reported by some studies²⁰, making the degree of selection bias inherent in HPV+ PDX generation unclear. When reported, HPV+ HNSCC engraftment rates have ranged widely from 14%¹⁸ to 100%²³, with highest rates in the series with very small numbers of tumors^{21, 23}. Minimal detail regarding patient selection^{15, 18} raised the possibility of bias introduced by specimens coming from highly atypical patients with bulkiest disease providing high tissue yield. In addition, some studies define engraftment based on initial growth in mice without subsequent *in vivo* passage^{21, 23} and thus fail to confirm long-term utility of the PDX. Lastly, variations in specimen handling, definition of HPV status, and engraftment methodology among studies obscure any barriers to HPV+ PDX generation and application.

Here these issues were addressed by applying uniform methodology over one year to generate PDXs from a practice performing a high volume of primary surgery for HPV+ disease. Differences in overall survival between HPV+ versus HPV- patients were consistent with those reported in a large prospective series²⁴, albeit with slightly higher mortality in the HPV- component of this much smaller cohort. In our cohort, PNI was independently associated with likelihood of engraftment and thus may introduce selection bias into PDX models for both HPV+ and HPV- groups. Lower rates of HPV+ versus HPV- tumor engraftment were also observed, resulting partly from frequent outgrowth of human EBV+ lymphomas in the HPV+ group. Despite lower yields of HPV+ PDXs suggesting greater selection bias in the HPV+ models generated, these PDXs retained hallmark morphologic and molecular traits of the disease subtype that were maintained during subsequent passages. These results establish their long-term value as research tools despite the resource intensive nature of generating these models based on their low engraftment rates.

Although HPV+ PDXs retained hallmark features of the disease, the low HPV+ PDX yield in this study likely produced certain molecular selection biases. Selection biases arising from low engraftment rates have been described for PDX models of breast cancer, which form at higher frequency from tumors with particular molecular traits^{25, 26}. Similar effects may arise in HPV+ PDXs based on a reduced burden of cancer-related mutations in these tumors relative to HPV- disease^{5–7}. Another type of molecular bias was previously shown in HNSCC PDXs using reverse phase protein arrays, where altered expression of certain

proteins in PDXs can cause them to resemble each other over their tumors of origin²¹. Ongoing whole exome and transcriptome analysis of our PDX panel will divide it into molecular subgroups and delineate its biases relative to the molecular description of HNSCC in the Cancer Genome Atlas.

Despite any concerns regarding selection bias, the large scale genetic alterations known to occur upon establishing feeder-independent cancer cell lines are largely absent during *in vivo* PDX engraftment^{13–16}. The fidelity of PDXs to their tumors of origin is however limited by slow genetic drift, which is often accompanied by accelerated growth with ongoing passage^{27, 28}. Live cryopreservation of large batches of PDX tissue from early passages is important to limit such effects and retain new PDXs as durable resources. PDXs also do not faithfully represent the human tumor microenvironment *in vivo* because they are devoid of human stroma including immune cells and fibroblasts, making them poor immunotherapy models. Efforts to address such limitations by reconstitution of PDX-bearing mice with HLA-matched human immune systems²⁹ are resource intensive and remain in their infancy. A slightly improved stromal environment for HNSCC PDXs may also be provided by orthotopic transplantation^{30–32}. Similarly, co-transplantation of human fibroblasts may provide more permissive growth conditions but has produced variable results in practice^{33, 34}.

The low engraftment in this study also arose in part from 24% of HPV+ tumors implanted producing xenografts of lymphoid and not epithelial origin. This frequent lymphoma outgrowth from HPV+ tumors may have been facilitated by co-transfer of the lymphoid-rich stroma from the tonsil and tongue base. Outgrowth of tumor types differing from the one implanted is a known phenomenon arising either from transfer of passenger human B- or T-cells or induction of murine-derived tumors at the implant site^{35, 36}. Contamination of HPV+ HNSCC specimens by their normal tissues of origin inevitably co-transfers tonsillar germinal center B-cells, which frequently harbor latent EBV infection^{37–39}. Accordingly, all aberrant tumors in this study were human EBV+ large B-cell lymphomas that likely expanded upon loss of immune surveillance in NSG mice in a phenomenon resembling post-transplant lymphoproliferative disease^{35, 40–42}. Thus FACS depletion or magnetic bead negative selection of lymphocytes from HPV+ HNSCCs prior to implantation might be a strategy to increase engraftment rates.

Given the paucity of HPV+ HNSCC cell lines, the potential for HPV+ PDXs to show feeder-dependent growth in short-term culture further underscores their utility for advancing basic and preclinical research. Such expansion of HPV+ HNSCC cells from PDXs using feeder cell-dependent primary culture would offer a renewable source of tumor cells for *in vitro* studies. Growth of such primary cultures has recently been aided by the ROCK inhibitor Y-27632, which can conditionally reprogram primary epithelial cells to suppress differentiation and proliferate indefinitely *in vitro*^{43, 44}. Using this methodology, HPV+ PDX-derived cells have shown strong proliferation in our recent pilot studies (data not shown), thus creating a platform to innovate *in vitro* experimental models of HPV+ disease. These emerging findings highlight the ability to establish *in vitro* primary culture models from HPV+ PDXs despite their relative resistance to feeder independence.

Conclusion

HPV-negative status and PNI are predictive clinical factors for PDX formation, whereas outgrowth of EBV+ lymphomas is a potential barrier to durable engraftment of HPV+ HNSCCs. Despite these limitations, 7 HPV+ PDXs were successfully established, including 5 from small, trans-orally resected tumors. Retention of key gross, pathologic, and molecular features by these PDXs highlights their value for studying this disease subtype and helps address the paucity of experimental models for HPV+ HNSCC.

Acknowledgments

Funding: This study was supported by NIH grants K08-DE022842 (D.B.), R21-DE024396 (D.B, H.N., P.A.G.), P01-CA098101 (D.B., P.A.G, H.N., A.K.R.), F32-DE024685 (N.F.), P30-DK050306 (Core Facilities), and the ACS/Triological Society Clinical Scientist Development Award (D.B.)

Bibliography

1. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst.* 2000; 92:709–720. [PubMed: 10793107]
2. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. *J Clin Oncol.* 2015; 33:3235–3242. [PubMed: 26351338]
3. Mallen-St Clair J, Alani M, Wang MB, Srivastan ES. Human papillomavirus in oropharyngeal cancer: The changing face of a disease. *Biochim Biophys Acta.* 2016; 1866:141–150. [PubMed: 27487173]
4. Castellsague X, Alemany L, Quer M, et al. HPV Involvement in Head and Neck Cancers: Comprehensive Assessment of Biomarkers in 3680 Patients. *J Natl Cancer Inst.* 2016; 108:djv403. [PubMed: 26823521]
5. Agrawal N, Frederick MJ, Pickering CR, et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science.* 2011; 333:1154–1157. [PubMed: 21798897]
6. Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science.* 2011; 333:1157–1160. [PubMed: 21798893]
7. Nichols AC, Palma DA, Chow W, et al. High frequency of activating PIK3CA mutations in human papillomavirus-positive oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg.* 2013; 139:617–622. [PubMed: 23787421]
8. Ndiaye C, Mena M, Alemany L, et al. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. *Lancet Oncol.* 2014; 15:1319–1331. [PubMed: 25439690]
9. Fakhry C, Westra WH, Li S, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *J Natl Cancer Inst.* 2008; 100:261–269. [PubMed: 18270337]
10. Bhatia A, Burtneess B. Human Papillomavirus-Associated Oropharyngeal Cancer: Defining Risk Groups and Clinical Trials. *J Clin Oncol.* 2015; 33:3243–3250. [PubMed: 26351343]
11. Zhao M, Sano D, Pickering CR, et al. Assembly and initial characterization of a panel of 85 genomically validated cell lines from diverse head and neck tumor sites. *Clin Cancer Res.* 2011; 17:7248–7264. [PubMed: 21868764]
12. Rygaard J, Povsen CO. Heterotransplantation of a human malignant tumour to "nude" mice. 1969. *APMIS.* 2007; 115:604–6. discussion 607–8. [PubMed: 17504422]
13. Daniel VC, Marchionni L, Hierman JS, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. *Cancer Res.* 2009; 69:3364–3373. [PubMed: 19351829]

14. Gillet JP, Calcagno AM, Varma S, et al. Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance. *Proc Natl Acad Sci U S A*. 2011; 108:18708–18713. [PubMed: 22068913]
15. Peng S, Creighton CJ, Zhang Y, et al. Tumor grafts derived from patients with head and neck squamous carcinoma authentically maintain the molecular and histologic characteristics of human cancers. *J Transl Med*. 2013; 11 198-5876-11-198.
16. Hidalgo M, Amant F, Biankin AV, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov*. 2014; 4:998–1013. [PubMed: 25185190]
17. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat Med*. 2015; 21:1318–1325. [PubMed: 26479923]
18. Klinghammer K, Raguse JD, Plath T, et al. A comprehensively characterized large panel of head and neck cancer patient-derived xenografts identifies the mTOR inhibitor everolimus as potential new treatment option. *Int J Cancer*. 2015; 136:2940–2948. [PubMed: 25404014]
19. Keysar SB, Astling DP, Anderson RT, et al. A patient tumor transplant model of squamous cell cancer identifies PI3K inhibitors as candidate therapeutics in defined molecular bins. *Mol Oncol*. 2013; 7:776–790. [PubMed: 23607916]
20. Anderson RT, Keysar SB, Bowles DW, et al. The dual pathway inhibitor rigosertib is effective in direct patient tumor xenografts of head and neck squamous cell carcinomas. *Mol Cancer Ther*. 2013; 12:1994–2005. [PubMed: 23873848]
21. Li H, Wheeler S, Park Y, et al. Proteomic Characterization of Head and Neck Cancer Patient-Derived Xenografts. *Mol Cancer Res*. 2016; 14:278–286. [PubMed: 26685214]
22. Basu D, Bewley AF, Sperry SM, et al. EGFR inhibition promotes an aggressive invasion pattern mediated by mesenchymal-like tumor cells within squamous cell carcinomas. *Mol Cancer Ther*. 2013; 12:2176–2186. [PubMed: 23939378]
23. Kimple RJ, Harari PM, Torres AD, et al. Development and characterization of HPV-positive and HPV-negative head and neck squamous cell carcinoma tumorgrafts. *Clin Cancer Res*. 2013; 19:855–864. [PubMed: 23251001]
24. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med*. 2010; 363:24–35. [PubMed: 20530316]
25. Charafe-Jauffret E, Ginestier C, Bertucci F, et al. ALDH1-positive cancer stem cells predict engraftment of primary breast tumors and are governed by a common stem cell program. *Cancer Res*. 2013; 73:7290–7300. [PubMed: 24142344]
26. Moon HG, Oh K, Lee J, et al. Prognostic and functional importance of the engraftment-associated genes in the patient-derived xenograft models of triple-negative breast cancers. *Breast Cancer Res Treat*. 2015; 154:13–22. [PubMed: 26438141]
27. Pearson AT, Finkel KA, Warner KA, et al. Patient-derived xenograft (PDX) tumors increase growth rate with time. *Oncotarget*. 2016; 7:7993–8005. [PubMed: 26783960]
28. Li S, Shen D, Shao J, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep*. 2013; 4:1116–1130. [PubMed: 24055055]
29. Reconstituting the Human Immune System. *Cancer Discov*. 2016; 6:812–8290. CD-ND2016-004. Epub 2016 Jul 12. [PubMed: 27406744]
30. Wang X, Fu X, Kubota T, Hoffman RM. A new patient-like metastatic model of human small-cell lung cancer constructed orthotopically with intact tissue via thoracotomy in nude mice. *Anticancer Res*. 1992; 12:1403–1406. [PubMed: 1332576]
31. Dong X, Guan J, English JC, et al. Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. *Clin Cancer Res*. 2010; 16:1442–1451. [PubMed: 20179238]
32. Vidal A, Munoz C, Guillen MJ, et al. Lurbinectedin (PM01183), a new DNA minor groove binder, inhibits growth of orthotopic primary graft of cisplatin-resistant epithelial ovarian cancer. *Clin Cancer Res*. 2012; 18:5399–5411. [PubMed: 22896654]

33. Ginestier C, Hur MH, Charafe-Jauffret E, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell*. 2007; 1:555–567. [PubMed: 18371393]
34. Zhang X, Claerhout S, Prat A, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. *Cancer Res*. 2013; 73:4885–4897. [PubMed: 23737486]
35. Bondarenko G, Ugolkov A, Rohan S, et al. Patient-Derived Tumor Xenografts Are Susceptible to Formation of Human Lymphocytic Tumors. *Neoplasia*. 2015; 17:735–741. [PubMed: 26476081]
36. Huang P, Westmoreland SV, Jain RK, Fukumura D. Spontaneous nonthymic tumors in SCID mice. *Comp Med*. 2011; 61:227–234. [PubMed: 21819692]
37. Perry M, Whyte A. Immunology of the tonsils. *Immunol Today*. 1998; 19:414–421. [PubMed: 9745205]
38. Assadian F, Sandstrom K, Bondeson K, et al. Distribution and Molecular Characterization of Human Adenovirus and Epstein-Barr Virus Infections in Tonsillar Lymphocytes Isolated from Patients Diagnosed with Tonsillar Diseases. *PLoS One*. 2016; 11:e0154814. [PubMed: 27136093]
39. Kuppers R. B cells under influence: transformation of B cells by Epstein-Barr virus. *Nat Rev Immunol*. 2003; 3:801–812. [PubMed: 14523386]
40. Chen K, Ahmed S, Adeyi O, Dick JE, Ghanekar A. Human solid tumor xenografts in immunodeficient mice are vulnerable to lymphomagenesis associated with Epstein-Barr virus. *PLoS One*. 2012; 7:e39294. [PubMed: 22723990]
41. Zhang L, Liu Y, Wang X, et al. The extent of inflammatory infiltration in primary cancer tissues is associated with lymphomagenesis in immunodeficient mice. *Sci Rep*. 2015; 5:9447. [PubMed: 25819560]
42. John T, Yanagawa N, Kohler D, et al. Characterization of lymphomas developing in immunodeficient mice implanted with primary human non-small cell lung cancer. *J Thorac Oncol*. 2012; 7:1101–1108. [PubMed: 22617243]
43. Liu X, Ory V, Chapman S, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am J Pathol*. 2012; 180:599–607. [PubMed: 22189618]
44. Palechor-Ceron N, Supryniewicz FA, Upadhyay G, et al. Radiation induces diffusible feeder cell factoRs) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells. *Am J Pathol*. 2013; 183:1862–1870. [PubMed: 24096078]

Figure 1. Application of a uniform PDX generation protocol to a clinical cohort of HNSCCs
(A) Workflow for PDX library generation with criteria for successful establishment. **(B)** Clinical features of HPV+ vs. HPV- patient subgroups. **(C)** Kaplan-Meier survival curves for HPV+ vs. HPV- HNSCC patients in the study (n=45).

Figure 2. HPV negative status and perineural invasion independently predict PDX engraftment
Engraftment rates HNSCC tumors (n=42) based on subgroups defined by HPV status and the presence or absence of PNI.

Figure 3. EBV+ B-cell lymphoma formation from a HPV+ HNSCC

Micrographs show a representative HPV+ primary tumor and the non-epithelial xenograft derived from it. IHC staining detects pan-cytokeratin, CD20, and CD3. ISH is shown for EBER1. (20x, Bar=50µm)

Figure 4. PDXs maintain histologic and molecular traits of HPV+ oropharyngeal cancer
(A) Gross images of HPV+ PDXs depict a spectrum of cystic architecture (top), and corresponding H&Es illustrate tumor walls encapsulating cysts (bottom). (4x, Bar=50µm)
(B) QRT-PCR for HPV-16 E6 and E7 viral transcript expression, where scatter plot illustrates Ct values for E6 and E7 in HPV+ vs. HPV- PDXs.

Table 1

Univariate analysis of engraftment success vs. clinical and pathological factors.

	n	% Engrafted	p-value*	n	% Engrafted	n	% Engrafted
T Stage			0.101				
T1/T2	24	20.8		20	20.0	4	25.0
T3/T4	18	44.4		7	14.3	11	63.6
Overall Stage			0.868				
Early	8	37.5		4	50.0	4	25.0
Advanced	34	29.4		23	13.0	11	63.6
Smoking			0.773				
Never &							
<10	18	33.3		14	21.4	4	75.0
10	24	29.2		13	15.4	11	45.5
Absent	21	14.3		15	6.7	6	33.3
Present	21	47.6		12	33.3	9	66.7
Lymphovascular Invasion			0.936				
Absent	23	30.4		15	20.0	8	50.0
Present	19	31.6		12	16.7	7	57.1
Extracapsular Extension			0.470				
Absent	19	21.1		14	14.3	5	40.0
Present	14	35.7		8	12.5	6	66.7
NA		44.4		5	40.0	4	50.0
Neg	15	53.3		---	---	15	53.3
Pos	27	18.5		27	18.5	---	---

* p-value for chi-square test or Fisher's exact test comparing success rates within subgroups.