Head and neck squamous cell carcinomas (HNSCC), which arise from the mucosal lining of the oral cavity, pharynx, and larynx, are increasing in incidence worldwide (1). Risk factors for HNSCC include smoking and alcohol consumption, as well as exposure to human papillomavirus (HPV; ref. 2). Analysis of the mutational landscape of HNSCCs (3) has not revealed druggable targets for the cancer, and current therapy entails, in many cases, a complex combination of surgery, radiation, and chemotherapy. Aggressive treatment, sometimes entailing induction chemotherapy (4), can be curative, but relapse rates may exceed 50%. Immunotherapy has also recently emerged as another modality for HNSCC, and some successful studies with immune checkpoint therapy have been reported.

Given the heterogeneity of HNSCCs, in terms of their drug responsiveness and aggressive potential, there is a vexing need for reliable model systems, which can identify treatment strategies for individual patients. Through the successful development of three-dimensional culture techniques and advanced growth factor supplementation, organoid models have been established recently for a wide range of human epithelial cancers (5, 6). Organoids are generated from fresh, viable tissue samples from human tumors. These models are faster, easier, and less expensive to generate than patient-derived xenograft (PDX) mouse models. They often uncover clonal heterogeneity of tumors and can be generated without xenotransplantation, and novel drug and conventional chemotherapy/radiation (chemo/RT) testing.

A previous study from another laboratory group (10) described culture conditions for HNSCC organoids and partially characterized the organoids with respect to \textit{ex vivo} drug sensitivity and tumorigenicity. The new study by Driehuis and colleagues significantly extends this earlier work. It describes culture conditions for organoids from normal human stratified mucosal lining epithelial cells or HNSCC tumor cells (8). The organoid culturing was rapid, with successful passage within 10–14 days of \textit{ex vivo} growth, and it exceeded 65% efficiency. This high success rate for HNSCC, compared with the lower efficiency of organoid generation from other tumor types such as prostate and pancreatic cancers, may result in part from the relatively easier access of larger tumor biopsies from head and neck tumors. The rapid outgrowth and high efficiency of HNSCC organoids, which is also observed for high-grade serous ovarian tumor organoids (9), may result, at least in part, from their underlying p53 mutations (the most common genetic mutation in these cancers). This intrinsic rapid cellular growth rate for HNSCC or ovarian cancer organoids may allow immune therapy testing in the organoid cultures with the tumor-infiltrating lymphocytes from the tumor. Importantly, Driehuis and colleagues were able to subject these organoids to a battery of novel functional assays not used previously in other organoid publications. Among the assays employed, the investigators used mutation and transcriptional profiling, chromosome stability, xenotransplantation, and novel drug and conventional chemotherapy/radiation (chemo/RT) testing.

In this issue, Driehuis and colleagues provide the first detailed description of culture conditions for organoids from normal human stratified mucosal lining epithelial cells or from HNSCC tumor cells (8). The organoid culturing was rapid, with successful passage within 10–14 days of \textit{ex vivo} growth, and it exceeded 65% efficiency. This high success rate for HNSCC, compared with the lower efficiency of organoid generation from other tumor types such as prostate and pancreatic cancers, may result in part from the relatively easier access of larger tumor biopsies from head and neck tumors. The rapid outgrowth and high efficiency of HNSCC organoids, which is also observed for high-grade serous ovarian tumor organoids (9), may result, at least in part, from their underlying p53 mutations (the most common genetic mutation in these cancers). This intrinsic rapid cellular growth rate for HNSCC or ovarian cancer organoids may allow immune therapy testing in the organoid cultures with the tumor-infiltrating lymphocytes from the tumor. Importantly, Driehuis and colleagues were able to subject these organoids to a battery of novel functional assays not used previously in other organoid publications. Among the assays employed, the investigators used mutation and transcriptional profiling, chromosome stability, xenotransplantation, and novel drug and conventional chemotherapy/radiation (chemo/RT) testing.

A previous study from another laboratory group (10) described culture conditions for HNSCC organoids and partially characterized the organoids with respect to \textit{ex vivo} drug sensitivity and tumorigenicity. The new study by Driehuis and colleagues significantly extends this earlier work. It describes robust long-term culture conditions for HNSCC cells in a well-defined medium, and shows, by histologic and genetic comparison, that the organoid cells are indeed tumor-derived and not overgrown wild-type cells. Accordingly, the organoids described by Driehuis and colleagues are amenable to a wider range of functional analyses, including but not limited to drug screening, and offer the possibility of predicting drug response of individual patients with HNSCC in the clinic.
The new study by Driehuis and colleagues describes a battery of novel functional assays as well as novel applications of organoid models. First, the organoids derived from normal mucosal lining cells were used to model herpes simplex virus and HPV infection, providing a clear advantage over previous studies employing immortalized epithelial cell lines. Second, the HNSCC cells from individual patients could be expanded long-term and cryopreserved without loss of cell viability. Third, tumor organoids were exposed to a wide range of clinically relevant drugs or radiation treatments, thereby revealing the heterogeneous sensitivities of HNSCC tumor cells from individual patients. In some cases, drug sensitivities correlated with specific tumor-derived mutations, thus highlighting the potential application of the model system in clinical decision-making. Fourth, in anecdotal cases, the organoid drug responses correlated with the clinical response of 7 patients with HNSCC.

Perhaps the most interesting and novel part of the new work is the observed correlation between the radiotherapy sensitivity of the HNSCC organoids and the clinical response of the corresponding patient with HNSCC. Previous studies have demonstrated that organoids can partially predict the clinical response of metastatic gastrointestinal cancers to chemotherapy or to targeted therapy (11). But this new work by Driehuis and colleagues extends the use of tumor organoids to the prediction of clinical radiotherapy response. The results also confirm the ability of some drugs such as cisplatin to sensitize tumor cells to radiation. The work also underscores the importance of testing whole panels of drugs in the organoids, including the conventional drugs for HNSCC such as cisplatin, docetaxel, and fluorouracil, as well as experimental targeted agents. Indeed, all of these drugs should be tested, even if there are no obvious genetic markers in the tumor that might indicate whether a targeted drug will be effective. For instance, in one case an organoid responded strongly to a PI3K inhibitor, even though there was no clear activating mutation in the PI3K gene in the organoid. Accordingly, in some cases the functional studies may be more informative than the genetic survey.

The authors stress that their study does not claim to prove the predictive potential of HSNCC-derived organoids in guiding therapy. Their study was aimed instead to establish the experimental basis for a future prospective clinical trial, which in itself will be a large endeavor. The mucosal lining organoid culture platform described in the study will allow the derivation and propagation of wild-type and cancer cells from individual patients with HNSCC. Matched, isogenic organoids can also be established from patients in the post-treatment setting, providing there is sufficient viable residual tumor tissue after treatment available for biopsy. Ideally, the organoid cultures will be adapted as a correlative biomarker platform for patients with HNSCC enrolled...
in a large prospective coclinical trial. For instance, patients could be enrolled in a trial using a conventional chemo/RT regimen in which wild-type tissue organoids, pretreatment tumor organoids, and post-treatment tumor organoids are established from their corresponding biopsy sites. Careful dissection of the fresh tissue by a trained pathologist is required to separate the normal tissue from the tumor tissue prior to establishing the organoids. Such a trial would offer several advantages. First, if the trial is successful, it could establish whether the chemo/RT sensitivity of the organoids indeed matches the chemo/RT sensitivity of the patient in the clinic. A scenario in which the pretreatment tumor organoid is resistant to the chemo/RT, and the patient exhibits a corresponding clinical resistance to the same combination, could lead to an appropriate change in therapy. Second, the establishment of post-treatment tumor organoids may allow the identification of molecular mechanisms of tumor resistance. Depending on the mechanism, these organoids may have an acquired sensitivity to another drug. In this case, post-treatment tumor organoids could provide the rationale for an alternative therapy. The drug-resistant organoids have added value because they will accelerate the development of new drugs and drug combinations.

In summary, Driehuis and colleagues have established the starting point for a prospective trial in which organoid chemo/RT response and patient clinical response can be directly compared. Establishing the predictive value of HNSCC organoid testing will require large numbers of patients. Moreover, such a trial must take into account a large number of clinical variables. First, the site of the primary tumor (say, oral cavity, pharynx, or larynx) or stage is likely to influence the functional activity of the corresponding organoids. Second, the tumors will have wide-ranging differences in genetic constitution. Accordingly, it is essential for each organoid culture to have genetic profiling, including mutational screening and transcriptional analysis. Combining the genetic profile of an organoid with its cellular phenotype (Fig. 1) may allow patients to be divided into smaller subsets which are more or less likely to respond to a specific therapy. Third, many relapsed tumors and even primary tumors are likely to exhibit clonal heterogeneity. Accordingly, multiple tumor biopsies from the same patient may yield organoid cultures with different functional attributes and different drug responses. The likelihood of clonal heterogeneity would mandate the use of combination therapy, which again can be easily tested with the tumor organoid platform. Despite these caveats, the establishment of an organoid platform for HNSCC is likely to lead to important advances in HNSCC diagnostics and treatment.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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