European Journal of Cancer 213 (2024) 115100

Contents lists available at ScienceDirect

European Journal of Cancer

journal homepage: www.ejcancer.com

Feasibility analysis of using patient-derived tumour organoids for treatment decision guidance in locally advanced head and neck squamous cell carcinoma

Anne-Sophie Fisch [a,1], Ana Pestana [a,b,c,1], Vanessa Sachse [a,c], Christian Doll [d], Elena Hofmann [d], Max Heiland [d], Theresa Obermueller [e], Jan Heidemann [e], Steffen Dommerich [f], Diana Schoppe [a], Simon Schallenberg [g], Iris Piwonski [g], Eric Blanc [h], Ingeborg Tinhofer [a,c,*]

[a] Department of Radiooncology and Radiotherapy, Translational Radiation Oncology Research Laboratory, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany
[b] Comprehensive Cancer Center, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany
[c] German Cancer Research Center (DKFZ), Heidelberg, and German Cancer Consortium (DKTK), partner site Berlin, Germany
[d] Department of Oral and Maxillofacial Surgery, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Augustenburger Platz 1, 13353 Berlin, Germany
[e] Department of Otorhinolaryngology, Campus Benjamin-Franklin, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Hindenburgdamm 30, 12203 Berlin, Germany
[f] Department of Otorhinolaryngology, Campus Mitte, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany
[g] Institut für Pathologie, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany
[h] Core Unit Bioinformatics, Berlin Institute of Health at Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt Universität zu Berlin, Charitéplatz 1, 10117 Berlin, Germany

ARTICLE INFO

Keywords:
Patient-derived organoids
Functional precision oncology
Radiosensitivity screens

ABSTRACT

Background: Current treatment for head and neck squamous cell carcinoma (HNSCC) involves surgery, radiotherapy, and chemotherapy. Despite aggressive multimodal approaches, tumour recurrence occurs in 40–60 % of cases, leading to poor survival outcomes. HNSCC lacks common genetic drivers for tailored therapies, and reliable biomarkers for treatment selection are scarce. We investigated the procedural requirements for incorporating drug- and radiosensitivity screens in patient-derived organoids (PDOs) within a clinical trial framework.
Patients and methods: Fresh tumour samples ($N = 198$) from 186 HNSCC patients were included. Success rates of organoid establishment were correlated with clinical and procedural parameters. Timelines for establishment of PDO cultures were determined, and their long-term growth potential assessed by serial passaging. Additionally, we conducted whole exome sequencing on matched tumour-organoid pairs. Three PDO models were employed to establish radiosensitivity assays.
Results: In total, PDO models displaying histomorphological features and genomic alterations of parental tumours were successfully established for 35 % of patient tumours. Success rates rose to 77 % for samples with a tumour cell content of 30 % or higher. Advanced patient age, prior radiotherapy, and delays in tissue processing were identified as negative predictors for engraftment. The estimated time interval needed for screens was compatible with PDO-guided selection of curative-intent radiotherapy regimens.
Conclusions: Our findings suggest that with high-quality samples and efficient tissue processing, PDO screens can be successfully performed in 77 % of HNSCC patients. Given the procedural challenges involved, future clinical trials aiming to the utility of PDOs for guiding treatment decisions should consider implementing centralised PDO screening.

* Correspondence to: Charité – Universitätsmedizin Berlin, Department of Radiooncology and Radiotherapy, Charitéplatz 1, 10117 Berlin, Germany.
E-mail address: ingeborg.tinhofer@charite.de (I. Tinhofer).
[1] Contributed equally.

https://doi.org/10.1016/j.ejca.2024.115100
Received 21 September 2024; Accepted 20 October 2024
Available online 28 October 2024

1. Introduction

Approximately 900,000 individuals worldwide are annually diagnosed with head and neck squamous cell carcinomas (HNSCC), and nearly half of them succumb to the disease [1]. Commonly employed treatment modalities for HNSCC include a combination of surgery, radiotherapy, and chemotherapy. Despite multimodal treatment approaches that strongly impact quality of life, 40–60 % of the tumours recur with dismal survival outcomes for this patient group [2].

Unlike in HNSCC, where treatment recommendations mainly rely on tumour localisation, disease stage, and pathological risk factors, treatment standards in many other solid cancer types have shifted towards a more personalised approach based on molecular tumour profiling. In this context, patients undergo treatment with molecular-targeted agents tailored to the molecular tumour characteristics. This approach is particularly successful in patients with oncogene-driven lung cancers [3]. However, the scarcity of genomic drivers in HNSCC [4] rarely permits the alignment of patients with efficacious targeted therapies. Additionally, there is currently a lack of robust biomarkers for optimal selection of treatment [5]. Therefore, complementary strategies designed to address the current limitations of genomics-based precision oncology in HNSCC are needed to advance the field.

The use of personalised models that reflect the biological characteristics of individual tumours is emerging as a strategy to complement the use of genomics [6,7]. This methodology involves obtaining tumour tissue from patients, either through biopsy sampling or surgical procedures, and culturing it in *ex vivo* cell culture models such as organoids. Subsequently, these models can be leveraged for radiosensitivity and drug screening, with the findings guiding treatment selection. Indeed, pilot studies with limited patient numbers have already demonstrated a correlation between patient-derived organoid (PDO) responses to both radiation and drugs and the corresponding responses observed in patients [8–12]. While the idea of integrating functional tumour-specific assays into standard clinical practice is appealing in theory, there are practical hurdles to overcome. One major challenge is time constraints, especially for patients with locally advanced disease who require prompt initiation of therapy. In this study, our aim was to determine the critical procedural factors for utilising PDOs in clinical trials to evaluate their potential in guiding treatment decisions in HNSCC. Our assessment focused on determining the overall success rates of PDO generation within realistic timelines for conducting clinically applicable *ex vivo* radiosensitivity and drug screening. Additionally, we identified clinical and procedural factors that need to be considered in clinical trial protocols integrating PDO screens.

2. Patients and methods

2.1. Patients

Patients with a suspicious mucosal lesion or a histologically proven squamous cell carcinoma in the oral cavity, oropharynx, hypopharynx or larynx, or a carcinoma of unknown primary (CUP) were approached for sample donation. Patients who provided informed consent to a biobanking protocol approved by the local Institutional Review Board of Charité University Medicine, Germany (EA2/152/10) were included in this prospective study. Tumour tissue samples were collected either during the diagnostic biopsy procedure or the curative-intent surgery. Clinical follow-up information was collected in the institutional clinical cancer registry.

2.2. Study objective and endpoints

The study aimed to assess the feasibility of integrating tumour organoid cultures from HNSCC patients in a clinical trial. The primary endpoint was the proportion of treatment-naïve patients for whom tumour organoids could be expanded prior to the initiation of curative-intent radio(chemo)therapy. Secondary endpoint was the identification of clinical and procedural factors influencing success rates of generation and expansion of PDO cultures for radiosensitivity and drug screens.

2.3. Tissue processing

Tissue samples were immediately transferred into a tube containing RPMI1640 medium (Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) complemented with 2.5 % penicillin/streptomycin (Sigma-Aldrich, St.Louis, MO, USA) and 1 % gentamicin (Thermo Fisher Scientific), and stored in the refrigerator until transport to the laboratory for further processing. Processing was performed on ice. Tissue was cut into small pieces (~1mm^3), and enzymatically digested in tissue dissociation mix, consisting of Advanced DMEM/F12 medium (Gibco™) complemented with DNAse I (100 µg/ml, VWR International, Radnor, PA, USA), Dispase (100 µg/ml, Corning®, Corning, NY, USA) and Y-27632 dihydrochloride (10 µmol/L, Abmole, Houston, TX, USA). After incubation at 37 °C for 30 min, enzymatic dissociation was stopped on ice by adding basal medium, consisting of Advanced DMEM/F12 (1X), HEPES (1mol/L, Sigma-Aldrich), GlutaMax (1 %, Gibco™), N-Acetyl-L-cystein (1 mmol/L, Sigma-Aldrich), penicillin/streptomycin (1 %), gastrin (10 nmol/L, Sigma-Aldrich) and Y-27632 dihydrochloride (5 µmol/L). Suspension was strained over 500-µm and 40-µm filters (pluriSelect®), and centrifuged at 300 g for 5 min at 4 °C. Fragments ranging from > 40 µm to < 500 µm in size were employed for organoid cultures.

2.4. Organoid cultures

Tissue fragments were plated in cell culture plates (Corning®) and covered with an organoid medium (herein referred to PDO complete+2 medium) initially formulated by Driehuis et al. [8] and further optimised during the pilot phase of this project. PDO complete+ 2 medium consisted of basal medium supplemented with serum-free B-27 (1X, Gibco™), N2 (1X, Gibco™), recombinant human FGF basic (20 ng/ ml, PeproTech, Cranbury, NJ, USA), recombinant human EGF (50 ng/ml Invitrogen, Carlsbad, CA, USA), Y-27632 dihydrochloride (10 µmol/L), TGF-ß RI kinase-inhibitor VI, SB431542 (0.5 µmol/L, Sigma-Aldrich) and conditioned medium from the cell line CRL-2376™ (1:100, ATCC, Manassas, VA, USA) containing Wnt-3A, R-spondin and Noggin. Amphotericin B (250 µg/ml, Sigma-Aldrich) was added only for the first three days of culture. Cultures were kept at 37 °C and 5 % pCO2 in a humidified incubator. At 80 % confluence, cells were passaged with TrypLE™ Express Enzyme Phenol Red (1x, Thermo Fisher Scientific). Two-thousand cells were seeded in 15 µl of Matrigel (Corning®). Drops solidified at 37 °C for 30 min with the plate upside down before prewarmed PDO complete+ 2 medium was added. Medium was exchanged every 3–4 days. For passaging, Matrigel was scratched from the plate, transferred to a microcentrifuge tube and washed with cold Dulbecco's Phosphate Buffered Saline (Thermo Fisher Scientific). Organoids were then incubated for 2–5 min in TrypLE™ before reseeding. Alternatively, cells were resuspended in Recovery™ cell culture freezing medium (Thermo Fisher Scientific) for cryopreservation in liquid nitrogen.

2.5. Histopathological and molecular characterisation

At a minimum size of 100 µm, organoids were harvested for immunohistochemical analysis and molecular profiling by whole exome sequencing (WES). Detailed information on the applied protocols and bioinformatics analysis are provided in the supplementary information.

2.6. Radiosensitivity assays

One hundred cells per 9 µl Matrigel were plated in six replicates per dose in white 96-well plates. Upon reaching 30–40 µm, organoids were subjected to X-ray irradiation using single doses of up to 8 Gy delivered by YXLON Maxishot (YXLON International GmbH, Hamburg, Germany)

at 0.89 Gy / min. After seven days, the medium was replaced with 100 µl of Cell-Titer-Glo® luminescence cell viability reagent per well (Promega, Fitchburg, WS, USA). The plates were shaken orbitally at 300 rpm for 30 min to facilitate Matrigel dissolution. Luminescence was measured using a plate reader (VICTOR Nivo multimode, PerkinElmer, Waltham, MA, USA).

In the 24-well assay, organoids were dissociated into single cells using TrypLE™ for 15–20 min. Subsequently, 1000 cells per 15 µl Matrigel were re-seeded in triplicates per dose in a new 24-well plate. After 9 to 10 days, organoids were counted per well, and survival fractions were calculated according to Nagle and coworkers [13]. Clonogenic survival curves, fitted by the linear-quadratic model, were generated using mean survival fractions derived from three independent experiments. Calculations were conducted with GraphPad Prism (version 8, GraphPad Software, Boston, Massachusetts, USA).

2.7. Statistical analysis

Descriptive statistics were employed to analyse organoid formation rates according to clinical and procedural characteristics. Differences between categorical groups were assessed using the Pearson's chi-squared test. Multivariate logistic regression was utilised to ascertain the independent impact of each parameter. The impact of organoid-forming capacity of tumours on overall survival was assessed using the Kaplan-Meier method. Differences between survival curves were determined using the log-rank test. Statistical analyses were conducted using SPSS Statistics software (version 29.0.1.1, IBM, Armonk, NY, USA). Two-sided tests were employed for all analyses, with the level of significance set at $P < 0.05$.

3. Results

3.1. Success rates of organoid establishment depend on the timeliness of tissue processing

From March 2019 to February 2023, 198 tumour tissue samples were collected from 186 patients. Patients across all tumour stages were included in the study. The primary site of tumour localisation was the oral cavity in the majority of cases (137/186 patients, 73.7 %). 82.8 % of samples were derived from primary tumours and 17.2 % from recurrences. Detailed patient characteristics are presented in Table 1.

In this study, patients undergoing routine treatment at three clinical sites across the city of Berlin were enroled, resulting in a delay of tissue processing by at least one day in 75 % of patients. Consequently, we investigated the impact of this delay on success rates. Only samples from treatment-naïve patients ($N = 164$) were included in this analysis (Fig. 1). Formation of organoids defined as viable dynamically growing three-dimensional structures occurred in 55 % and 53 % of samples when tissue processing was conducted on the same (day 0) or the following day (day 1) after tissue collection, respectively. The organoid formation rate significantly dropped to 32 % when processing commenced only $\geq$ 2 days after tissue collection (Fig. 1 A, Pearson $\chi 2$ test: $P = 0.002$). Despite its negative impact on organoid formation, a delay in tissue processing did not interfere with long-term growth potential in successful organoid cultures (Fig. 1 B, Pearson $\chi 2$ test: $P = 0.36$). Overall, a 35 % success rate was attained in cultivating organoids with long-term growth potential when tumour tissue was promptly processed following sampling.

3.2. Histopathological and molecular analysis of organoids

To confirm the tumour origin of organoids, we conducted histological and immuno-histochemical validation following the standard protocol used for diagnosing HNSCC. As shown in Supplementary Fig. S1, organoids exhibited the histomorphological traits of the original tumour tissue, which remained consistent across successive passages. Additionally, the organoids recapitulated features associated with tumour aggressiveness, such as high expression of p40, which was higher in parental tumour samples from areas of invasive tumour growth (Supplementary Fig. S2, middle panel) compared to dysplastic regions at the tumour margins (Supplementary Fig. S2, left panel), where the transition to more invasive characteristics had begun. Molecular

Table 1
Organoid establishment success rates based on clinical and procedural parameters.

	All		**Organoid formation**				***Pearson***	**Multivariate**		
			yes		**no**		***χ2 test***	**logistic regression**		
	N patients	N samples	N	%	N	%	*P value*	HR	95 % CI	*P value*
TOTAL	186	198	88	44.4	110	55.6				
Age, years (median, range)			65 (18–86)		69 (38–91)		***0.001***	0.96	0.93–0.98	**0.003**
Sex							*1.000*			
male	116	124	55	44.4	69	55.6		1.00		
female	70	74	33	44.6	41	55.4		1.12	0.58–2.14	0.738
Sublocalisation							*0.067*			
oral cavity	137	147	63	42.9	84	57.1		1.00		
oropharynx	30	31	19	61.3	12	38.7		3.61	0.85–15.24	0.081
larynx, hypopharynx or CUP	19	20	6	30.0	14	70.0		0.57	0.19–1.69	0.311
p16 Status							*0.234*			
p16- and p16+non-OPC	167	179	77	43.0	102	57.0		1.00		
p16+OPC	19	19	11	57.9	8	42.1		0.35	0.07–1.87	0.218
Stage							*0.881*			
I-II	64	69	30	43.5	39	56.5		1.00		
III-IV	122	129	58	45	71	55		1.00	0.52–1.91	0.998
Tx status at sampling							***0.015***			
Tx naive		164	80	48.8	84	51.2		1.00		
recurrence (no RTx)		11	4	36.4	7	63.6		0.71	0.18–2.80	0.620
recurrence (previous RTx)		23	4	17.4	19	82.6		0.18	0.05–0.61	**0.006**
Sample type							*0.327*			
Biopsy		51	26	51.0	25	49.0		1.00		
Surgical specimen		147	62	42.2	85	57.8		0.83	0.37–1.83	0.637
Tissue processing							*0.152*			
day 0		49	25	51.0	24	49.0		1.00		
day 1		108	50	46.3	58	53.7		0.84	0.39–1.80	0.652
day > 1		41	13	31.7	28	68.3		0.45	0.17–1.18	0.104

Abbreviations: CUP, carcinoma of unknown primary; OPC, oropharynx carcinoma; RTx, radiotherapy; Tx, therapy.

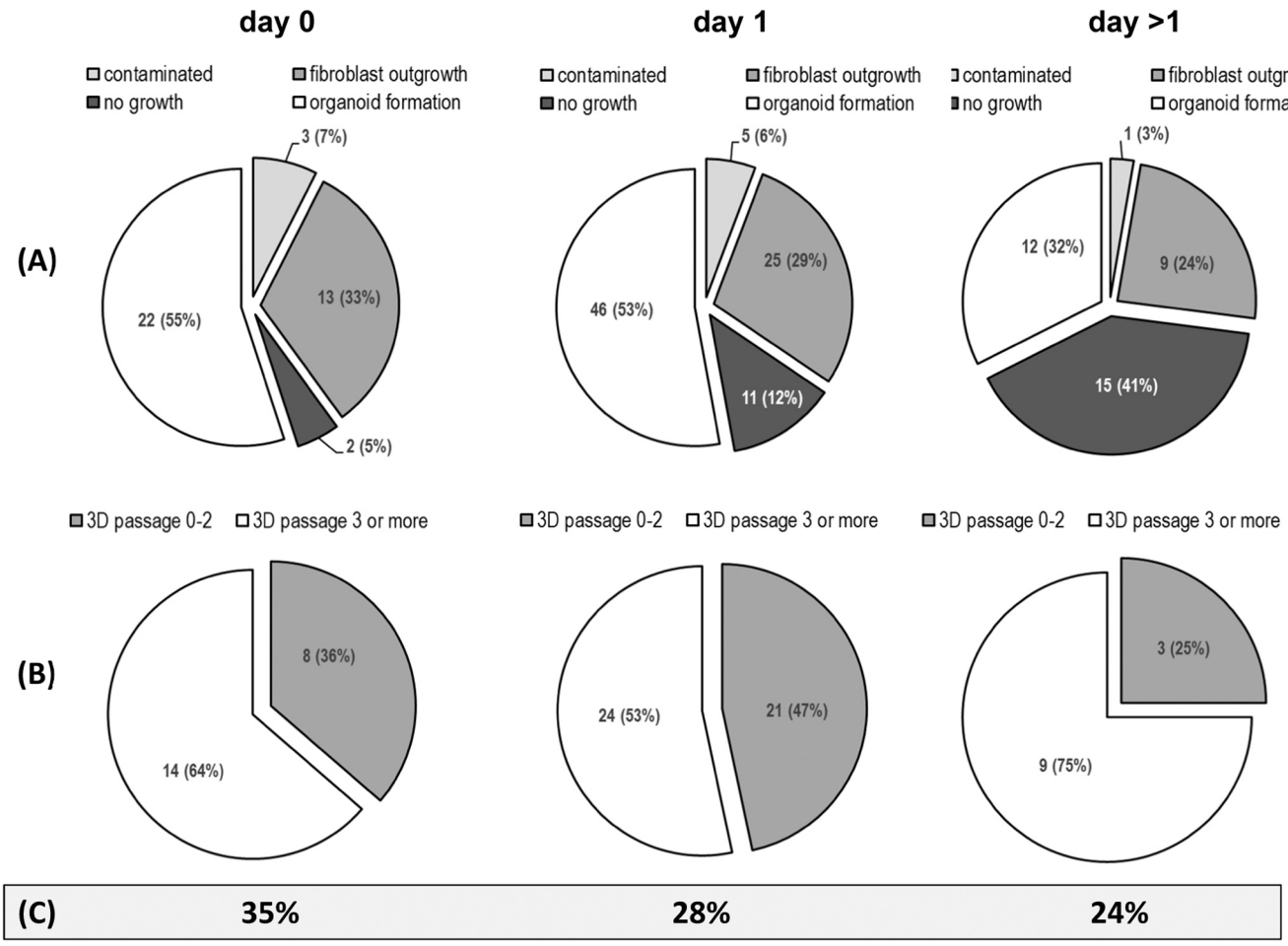

Fig. 1. Impact of delayed tissue processing on organoid forming rates and their long-term growth potential. (A) Tumour tissue samples from treatment-naïve patients were processed immediately (day 0), the next day (day 1), or two or more days (day >1) after collection. Pie charts display the percentages of successful cultures with organoid formation (□), as well as failed cultures due to microbial contamination (■), insufficient cellular growth (■) or fibroblast overgrowth (■). (B) Organoid cultures were divided into two groups based on their growth potential across serial passages, indicating stable growth for three or more passages (□) versus transient growth for up to two passages (■). (C) The overall success rates in establishing organoid cultures with sustained growth potential are presented. The number of samples and the relative proportions of subgroups are indicated in the pie charts.

analysis by WES was carried out on paired samples from parental tumour tissue and corresponding organoids for treatment-naïve tumours. To assess the impact of intratumour heterogeneity on the concordance between primary tumours and derived organoids, we included all available archival tumour tissue samples (mean: 3.3 samples per patient; range: 2–7). Out of 80 treatment-naïve cases with successful organoid engraftment, WES was successfully performed on 87 samples from 30 cases. The sample selection process for WES analysis is outlined in a flow chart in Supplementary Fig. S3. To mimic a potential clinical trial protocol of *ex vivo* radiosensitivity/drug screening in tumour organoids prior to commencing curative radiotherapy, we used the first passage of organoid cultures for mutational profiling. In the parental tumour samples (Supplementary Fig. S4), *TP53* mutations emerged as the most prevalent genetic alterations, detected in 27 out of the 30 cases (90 %). Additionally, mutations in *CDKN2A*, *NOTCH1*, *FAT1* and *CCND1* amplifications were frequently observed. Only 16 out of 30 (53 %) organoid cultures exhibited driver mutations, as annotated in OncoKB [14], consistent with those found in the original tumours, confirming their tumour origin (Fig. 2, left panel). Conversely, the absence of any matching driver mutation indicated normal organoid formation in the remaining 14 cases (47 %, Fig. 2, right panel). The inability of these models to sustain growth beyond two passages further supported their non-tumourigenic origin.

We postulated that the initial tumour cell content could be crucial for the successful formation of tumour organoids, with failures potentially stemming from insufficient tumour cell content in the original sample. Consequently, we categorized cases into three groups according to the allelic frequency of the key driver mutant variant ($TP53^{mut}$, n = 27; $BRCA1^{mut}$, n = 1; $CASP8^{mut}$, n = 1; $PIK3CA^{mut}$, n = 1; all annotated as oncogenic in OnkoKB™) in the patient's original tumour, serving as indicator of tumour cell content. Success rates surged from 28 % to 77 % as allelic frequencies of driver mutations in parental tumour tissue samples rose from < 0.15 to > 0.3 (Fig. 3), underscoring the critical importance of an initial sample quality check for achieving high success rates.

We then asked whether success rates depended not only on procedural factors and sample quality but also on patient and/or tumour characteristics. Univariate analysis revealed a significant association with patient age, with higher organoid formation rates in younger patients (Table 1). Furthermore, a trend to higher rates was observed for carcinomas of the oropharynx compared to other localisations. Establishment of organoids was significantly more often successful for tumour samples from treatment-naïve patients. Especially for patients previously treated with radiotherapy, the success rate dropped to 17 %. In contrast, no significant association was observed for sex, stage or p16 status (Table 1). Using a multivariate logistic regression model including patient age, sex, sub-localisation, p16 status, UICC stage, treatment status at sampling, the sampling type (biopsy or resection) and the time

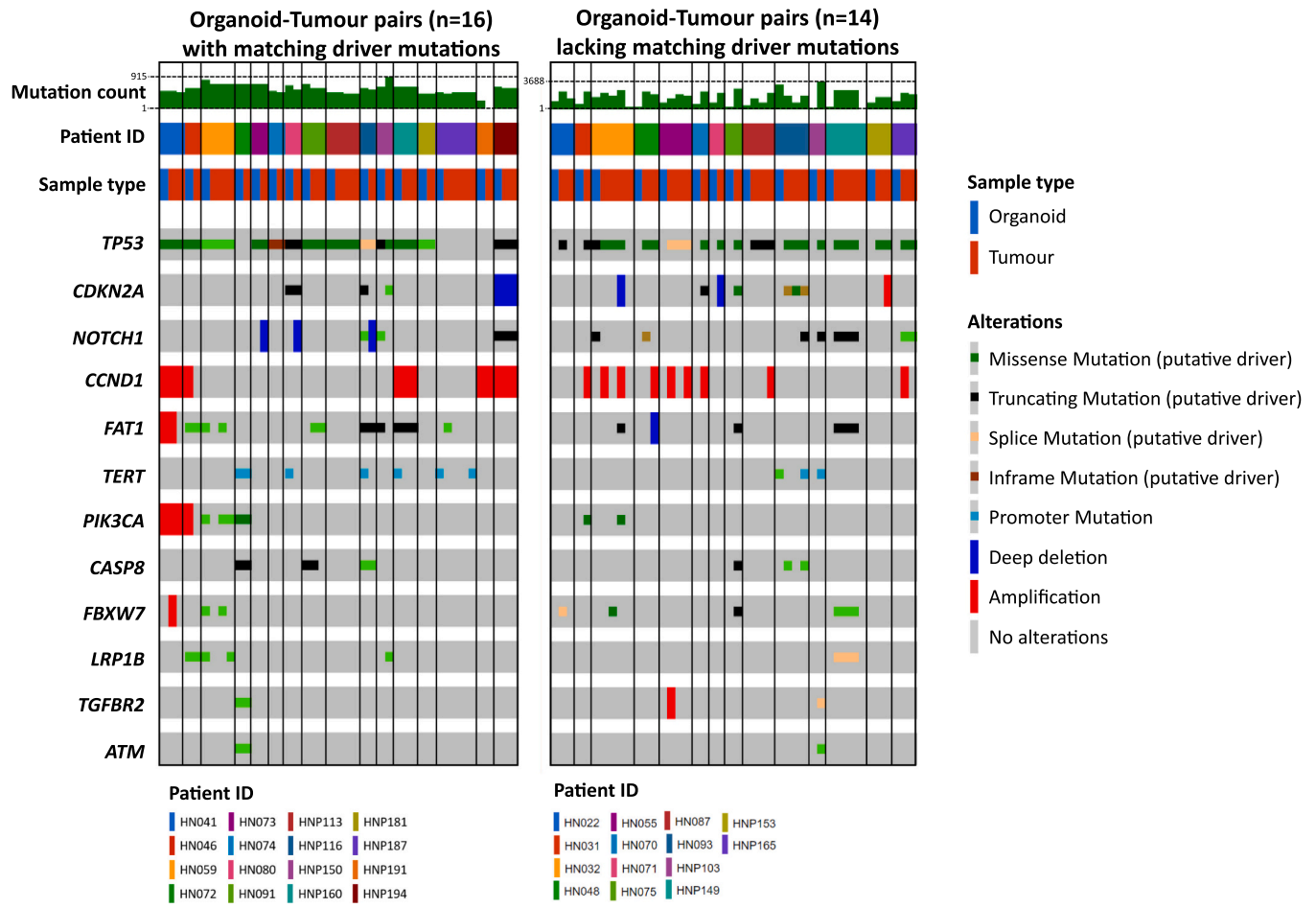

Fig. 2. Molecular analysis of organoids. Paired samples from 30 treatment-naïve patients, including parental tumour tissue and corresponding organoids, were subjected to WES analysis. The OncoPrint tool (cbioportal) was utilised for the visualisation of tumour mutational burden (TMB) and genomic alterations by heatmaps. The type of genetic alteration, patient IDs and sample types were colour-coded as indicated in the legend at the right and the bottom. Left panel: Organoid-Tumour pairs from $N = 16$ patients (number of analysed samples: $N = 27$ FFPE tumour samples; $N = 16$ organoid samples) with organoids matching driver mutations of parental tumours; right panel: Organoid-Tumour pairs from $N = 14$ patients (number of analysed samples: $N = 30$ FFPE tumour samples; $N = 14$ organoid samples) lacking driver mutations of parental tumours in the organoids.

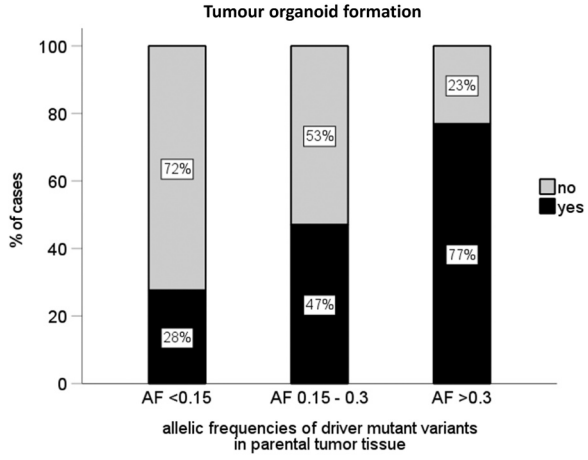

Fig. 3. Success rates of tumour organoids depend on tumour cell content in parental tumour tissue. Cases were categorized into three groups based on the allelic frequencies (AF) of driver mutant variants in the corresponding FFPE tumour tissue samples as a surrogate measure of tumour cell content. The relative numbers of *ex vivo* cultures with (black) or without (white) tumour organoid formation in the three categories (AF <0.15, AF 0.15–0.3, AF >0.3) are shown in a bar graph.

delay in tissue processing, we identified younger patient age, and a treatment-naïve status at sampling as the only independent factors associated with higher success rates (Table 1).

We were also interested whether organoid engraftment per se is a prognostic marker of poor outcome, as previously also reported for engraftment of HNSCC PDX models [15,16]. Only treatment-naïve patients from whom tissue samples were processed within day 0–1 and whose cultures were not compromised by bacterial contamination ($N = 119$) were included in this analysis. A trend to reduced overall survival was observed in the group of patients with successful organoid engraftment compared to patients from whom an *ex vivo* culture could not be established (supplementary Fig. S5).

3.3. Timelines for organoid establishment and radiosensitivity screens

We then calculated the median time required for organoid establishment and expansion for *ex vivo* screens (Fig. 4A) and determined the percentage of patients for whom successful organoid establishment and expansion were completed before the initiation of curative-intent definitive or adjuvant radio(chemo)therapy. Again, only patients from whom tissue samples were processed within day 0–1 were included in this analysis. As depicted in Fig. 4 B, organoid cultures would have been established and expanded in time in 21 out of 25 patients (84 %), with a median of 21 days (range: 6–69 days) remaining for comprehensive

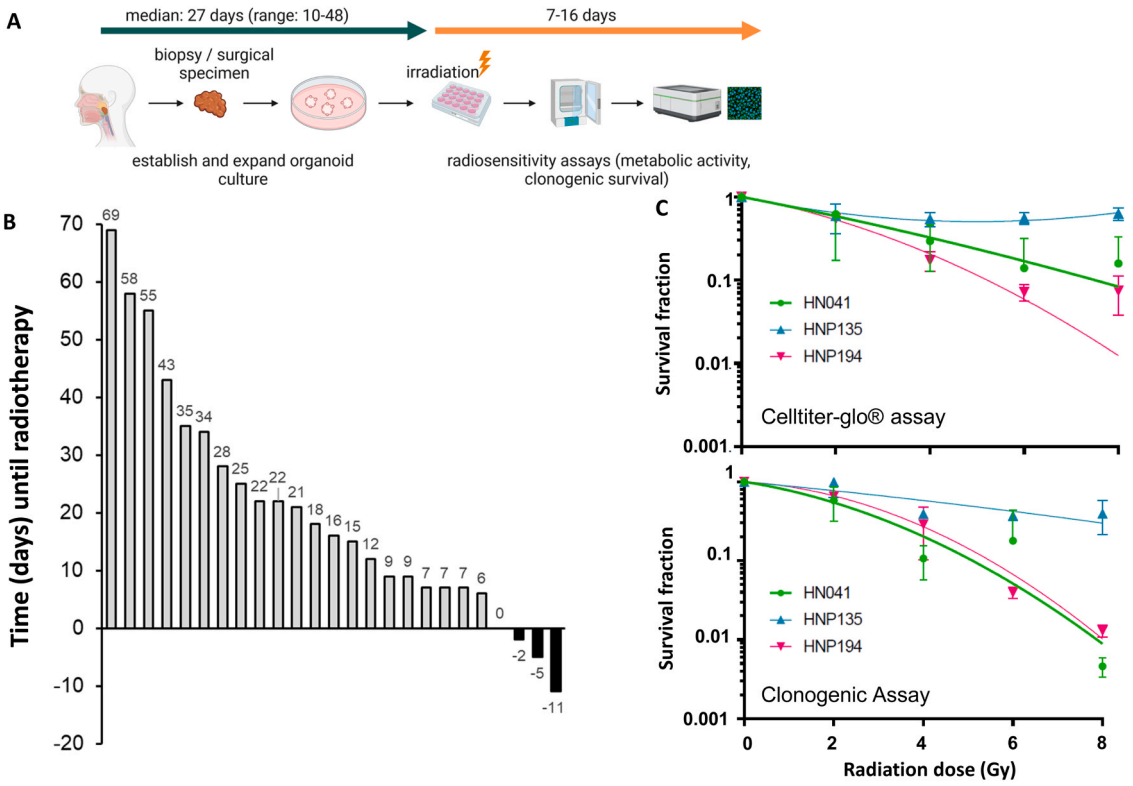

Fig. 4. Timelines for organoid establishment, expansion and radiosensitivity screens. (A) The diagram outlines the procedural workflow for *ex vivo* radiosensitivity analysis in PDOs, with indicated timeframes for each step. This visual representation was generated using BioRender.com. (B) The bars represent the duration (in days) between the establishment and expansion of organoid cultures and the commencement of radiotherapy (designated as day=0) for 25 patients receiving definitive or adjuvant radiotherapy. Positive values denote cases where establishment was completed before radiotherapy initiation (84 %), while negative values indicate cases where establishment occurred after (16 %). **(C)** Results from radiosensitivity testing of organoid models derived from one radiorefractory tumour (HNP135 from a male patient experiencing a second recurrence post re-irradiation of larynx carcinoma) and two radiosensitive tumours (HN041 from a treatment-naïve female patient with hypopharynx carcinoma; HNP194 from a treatment-naïve CUP patient) are presented, utilising either the Celltiter-glo® assay or clonogenic survival assay.

screening of potential drug-radiotherapy combinations. In a pilot experiment utilising organoid models derived from one radiorefractory (HNP135) and two radiosensitive tumours (HN041, HNP194), we confirmed that analysis of inherent radiosensitivity of tumours, using radiation-induced changes in metabolic activity and clonogenic survival as read-outs, is feasible. Importantly, clear differences in radiosensitivity of organoids from radiosensitive and radiorefractory tumours were observed (Fig. 4 C).

4. Discussion

We here show that PDO generation is achievable in the majority of HNSCC patients, provided that stringent measures are taken to ensure high sample quality and prompt tissue processing. Consistent with previous observations in patient-derived xenograft models [15,16], the success rates for organoid generation were particularly high in patients with aggressive tumours associated with poor outcomes. For this group of poor-prognosis patients, development of novel treatment approaches would be of utmost importance. The timelines achieved for PDO generation and expansion suggest their potential integration into clinical trials to assess their value in individualised treatment selection for these high-risk patients. Our data support the feasibility of incorporating PDO screens into such trials, provided sample logistics allow for shipment to a central laboratory within 24 h, where PDO establishment and screening can be efficiently carried out by a specialised team.

Higher patient age and prior radiotherapy were identified as negatively impacting the engraftment of tumour and non-tumour organoids. The stemness promoting conditions in organoid cultures favour the growth of normal and tumour stem cells [17]. A reduction of stem cells with aging [18] and as a side effect of irradiation [19] has been reported previously, potentially explaining the decreased organoid engraftment observed for HNSCC patients of higher age or radiotherapy pretreatment. Since a functional precision oncology-based treatment might be particularly beneficial in elderly patients unfit for standard multimodal treatment and HNSCC patients with recurrent disease, research efforts in the organoid field should prioritise understanding the mechanisms impairing organoid engraftment in these specific groups.

Inadequate engraftment rates would undoubtedly pose a significant obstacle to the clinical integration of PDOs for guiding individual treatment selection [20]. In the absence of an initial sample quality check, overall success rate of HNSCC organoid generation was only 35 % in our study. Similar rates for the establishment of head and neck cancer organoids have been reported in previous studies [11,21]. Assessing neoplastic cellularity by genomic sequencing should thus be considered an essential quality control before using tumour samples for organoid engraftment. This analytical prerequisite aligns with genomics-based precision oncology, where a tumour cell content of > 20 % in tumour tissue samples represents the threshold for high-quality molecular profiling [22]. Ideally, assessment of tumour cell content should be done intraoperatively, allowing re-sampling in the surgical theatre in case of insufficient sample quality. A promising new method for rapid intra-operative quantification of malignant cells in a tissue biopsy, using physical single-cell phenotyping, has recently been developed by Soteriou and colleagues [23]. Implementing this method in the sampling procedure could aid in increasing success rates for both molecular diagnostics and tumour organoid engraftment.

The interval between sample acquisition and tissue processing was another critical variable impacting success rates. Comparable engraftment outcomes were observed when tissue processing occurred within one or two days post-sampling, supporting the feasibility of organoid screens in multicentre clinical trial settings. However, success rates notably declined when processing commenced on the second day or later. Of note, we observed a heightened tolerance of the malignant cell population towards delayed tissue processing which was evidenced by sustained high growth rates in tumour organoid cultures initiated on day two or later. This aligns with findings indicating neoplastic cells' resilience to adverse conditions, such as overnight enzymatic tissue digestion, compared to normal cells – a phenomenon leveraged to enhance tumour organoid engraftment rates [24,25].

In our study, overgrowth of normal epithelial organoids was observed in one-third of the tumour samples. This phenomenon has been reported as a common issue in various cancer types [17,25–28]. It has been associated with optimised culture conditions that favour the proliferation of normal cells over tumour cells [29]. One approach to address this limitation is the addition of the Mdm2 antagonist Nutlin3a to the culture medium which induces p53-mediated cell cycle arrest and apoptosis in cells harbouring *TP53* wild-type [8]. Given the high frequency of *TP53* mutations in HNSCC, this strategy is anticipated to significantly enhance tumour organoid establishment. Despite being applicable only to *TP53* mutated cases, the pressing need for improving current treatment regimens for these tumours underscores the clinical relevance of this approach.

More than 45 % of HNSCC cases harbour mutations that aberrantly activate the Wnt signalling pathway [4], presenting a further opportunity to selectively expand tumour organoids [30]. This can be accomplished by using suboptimal culture media lacking stemness-maintaining factors like Wnt-3a. In such conditions, normal epithelial stem cells relying on external Wnt signalling are depleted, while tumour organoids with intrinsic Wnt activation are preserved. This strategy has demonstrated successful in colorectal [29] and lung cancer [17]. Own preliminary data from an HNSCC organoid model cultured with or without Wnt-activating factors (HN059, Supplementary Fig. S6), and results from a previous study [8] suggest that this approach can effectively enrich for tumour organoids in head and neck cancer as well.

Promising results from case series in rectal [9,10] and head and neck cancer [8,12] suggest that analysing radiosensitivity of PDOs may predict individual patient responses to radiation. Despite the limited sample size, our data further support this assumption. However, slow organoid growth could potentially cause significant delays in *ex vivo* screens, surpassing the timeframe during which patients could benefit from functional testing. According to clinical guidelines, curative treatment should commence within four weeks after diagnosis, with adjuvant radiotherapy ideally starting within six weeks post-surgery [31]. By initially expanding tumour cells in 2D cultures, we managed to generate sufficient organoids for experimental radiation within 3 weeks. Future improvement in sample quality and logistics is anticipated to further accelerate this process. Importantly, the initial 2D culture did not compromise the representativeness of tumour organoids, as confirmed by WES analysis of organoids and matching patient tumours. Radiosensitivity screens conducted on established organoid cultures were then completed within a median of 19 days, and the results of screens corresponded to the clinical outcome. Thus, our workflow could enable radiation oncologists to tailor individual treatment strategies in a timely manner within the curative therapy setting.

Beside confirming the feasibility of incorporating PDO screens into clinical trials, we have established a large living biobank of primary HNSCC organoids, preserving the genotype and histological phenotype of patients' tumours. This biobank will serve as a valuable tool for future biomarker development and treatment optimisation programmes. Incorporation of a living biobank, allowing detailed molecular and functional studies in responders and non-responders, in future clinical trials will also significantly accelerate the development of novel therapeutic approaches.

Our study has several limitations that warrant consideration. Firstly, due to the small sample sizes of carcinomas at sites other than the oral cavity, the feasibility of organoid screens in other anatomical subgroups and HPV-driven oropharyngeal carcinomas remains unknown. Additionally, we did not control for a stable cooling chain in the sample logistics, which could potentially also influence organoid engraftment. Though not the primary aim of our study, our preliminary results from radiosensitivity screenings in three PDO models support previous findings of a predictive potential of organoids for individual responses to radiotherapy [8,11,12]. However, the small number of patient models tested in our study does not allow for a definite conclusion in this respect.

A general limitation of current organoid protocols is that the tumour microenvironment, including essential components such as stromal and immune cells, is lost during serial passaging. These elements can play a critical role in influencing treatment responses and resistance mechanisms. Without these complex interactions, organoids may not fully replicate how tumours respond to radiation and drugs. Novel approaches to integrate human tumour microenvironment components into patient-derived organoids could thus further enhance the utility of these models in drug and biomarker development.

5. Conclusions

Engraftment of tumour organoids in up to 80 % of patients, coupled with their timely expansion, underscores the preparedness of these models for inclusion in clinical trials. Ensuring a high tumour cell content in original tissue samples and establishing standardised sample logistics are pivotal to guarantee high success rates. Moreover, our observation of higher engraftment rates in patients with poor overall survival underscores the potential of these models for optimising treatment and unravelling resistance mechanisms. To fully explore the potential of HNSCC organoid models in guiding individual treatment selection, particularly in the context of radiosensitization, future studies will need to include a larger number of HNSCC organoids and evaluate a broader panel of candidate drugs, both standard and novel, with potential radiosensitizing activity.

Authors' contributions

IT, ASF and AP designed the study. ASF, AP, VS, and DB performed the experiments. S.S. and I.P. performed the histopathological analysis. EH, CD, SD, TO, JH and MH recruited patients and collected clinical data. IT and ASF performed the statistical analyses. EB performed the bioinformatics analysis. IT, ASF, AP, VS, EB, SS, IP, CD, EH, TO, SD, MH and JH analysed and discussed the results. IT and ASF jointly developed the structure and arguments for the paper. IT, ASF, and VS wrote the manuscript. All authors discussed, made critical revisions and approved the final manuscript.

CRediT authorship contribution statement

Ingeborg Tinhofer: Conceptualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Steffen Dommerich:** Resources, Writing – review & editing. **Jan Heidemann:** Resources, Writing – review & editing. **Theresa Obermueller:** Resources, Writing – review & editing. **Eric Blanc:** Formal analysis, Writing – review & editing. **Ana Pestana:** Conceptualization, Data curation, Formal analysis, Writing – review & editing. **Iris Piwonski:** Data curation, Writing – review & editing. **Anne Sopie Fisch:** Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Simon Schallenberg:** Data curation, Writing – review & editing. **Diana Schoppe:** Data curation, Formal analysis, Writing – review & editing. **Max Heiland:** Resources, Writing –

review & editing. **Elena Hofmann:** Resources, Writing – review & editing. **Christian Doll:** Resources, Writing – review & editing. **Vanessa Sachse:** Data curation, Formal analysis.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT version 3.5 in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding

The work was supported by the Federal Ministry of Education and Research (BMBF), as part of the National Research Initiatives for Mass Spectrometry in Systems Medicine (grant agreement number 16LW0239K), and by the ERA PerMed Initiative (grant agreement number 01KU201). In addition, financial support was provided by an intramural fund of the German Cancer Consortium (DKTK) partner site Berlin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We extend our gratitude to all the patients who participated in this study, along with the clinical teams at each surgical clinic for their assistance in patient recruitment and tissue collection. Our appreciation also goes to Anna Happe-Kramer for her invaluable technical support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.115100.

References

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024.
[2] Mody MD, Rocco JW, Yom SS, et al. Head and neck cancer. Lancet 2021;398 (10318):2289–99.
[3] Passaro A, Al Bakir M, Hamilton EG, et al. Cancer biomarkers: Emerging trends and clinical implications for personalized treatment. Cell 2024;187(7):1617–35.
[4] Cancer Genome Atlas Network.. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517(7536):576–82.
[5] Budach V, Tinhofer I. Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: a systematic review. Lancet Oncol 2019;20(6): e313–26.
[6] Kornauth C, Pemovska T, Vladimer GI, et al. Functional precision medicine provides clinical benefit in advanced aggressive hematologic cancers and identifies exceptional responders. Cancer Discov 2022;12(2):372–87.
[7] van Renterghem AWJ, van de Haar J, Voest EE. Functional precision oncology using patient-derived assays: bridging genotype and phenotype. Nat Rev Clin Oncol 2023;20(5):305–17.
[8] Driehuis E, Kolders S, Spelier S, et al. Oral mucosal organoids as a potential platform for personalized cancer therapy. Cancer Discov 2019;9(7):852–71.
[9] Yao Y, Xu X, Yang L, et al. Patient-derived organoids predict chemoradiation responses of locally advanced rectal cancer. Cell Stem Cell 2020;26(1). : 17-26.e16.
[10] Hsu KS, Adileh M, Martin ML, et al. Colorectal cancer develops inherent radiosensitivity that can be predicted using patient-derived organoids. Cancer Res 2022;82(12):2298–312.
[11] Millen R, De Kort WWB, Koomen M, et al. Patient-derived head and neck cancer organoids allow treatment stratification and serve as a tool for biomarker validation and identification. Med 2023;4(5). : 290-310 e212.
[12] Choi SY, Shim J, Gu DE, et al. Clonal evolution of long-term expanding head and neck cancer organoid: Impact on treatment response for personalized therapeutic screening. Oral Oncol 2023;146:106571.
[13] Nagle PW, Hosper NA, Ploeg EM, et al. The in vitro response of tissue stem cells to irradiation with different linear energy transfers. Int J Radiat Oncol Biol Phys 2016;95(1):103–11.
[14] Chakravarty D, Gao J, Phillips SM, et al. OncoKB: a precision oncology knowledge base. JCO Precis Oncol 2017;2017.
[15] Karamboulas C, Bruce JP, Hope AJ, et al. Patient-derived xenografts for prognostication and personalized treatment for head and neck squamous cell carcinoma. Cell Rep 2018;25(5). : 1318-1331 e1314.
[16] Klinghammer K, Otto R, Raguse JD, et al. Basal subtype is predictive for response to cetuximab treatment in patient-derived xenografts of squamous cell head and neck cancer. Int J Cancer 2017;141(6):1215–21.
[17] Kim M, Mun H, Sung CO, et al. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. Nat Commun 2019;10(1):3991.
[18] GAd Santos, Magdaleno GDV. Magalhães JPd. Evidence of a pan-tissue decline in stemness during human aging. bioRxiv 2023;2023. 2004.2013.536766.
[19] Sonis ST. A hypothesis for the pathogenesis of radiation-induced oral mucositis: when biological challenges exceed physiologic protective mechanisms. Implications for pharmacological prevention and treatment. Support Care Cancer 2021;29(9):4939–47.
[20] Ooft SN, Weeber F, Schipper L, et al. Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. ESMO Open 2021;6 (3):100103.
[21] Tanaka N, Osman AA, Takahashi Y, et al. Head and neck cancer organoids established by modification of the CTOS method can be used to predict in vivo drug sensitivity. Oral Oncol 2018;87:49–57.
[22] Mock A, Teleanu M-V, Kreutzfeldt S, et al. NCT/DKFZ MASTER handbook of interpreting whole-genome, transcriptome, and methylome data for precision oncology. npj Precis Oncol 2023;7(1):109.
[23] Soteriou D, Kubánková M, Schweitzer C, et al. Rapid single-cell physical phenotyping of mechanically dissociated tissue biopsies. Nat Biomed Eng 2023;7 (11):1392–403.
[24] Driehuis E, Gracanin A, Vries RGJ, et al. Establishment of pancreatic organoids from normal tissue and tumors. STAR Protoc 2020;1(3):100192.
[25] Broutier L, Mastrogiovanni G, Verstegen MMA, et al. Human primary liver cancer–derived organoid cultures for disease modeling and drug screening. Nat Med 2017;23(12):1424–35.
[26] Goldhammer N, Kim J, Timmermans-Wielenga V, et al. Characterization of organoid cultured human breast cancer. Breast Cancer Res 2019;21(1):141.
[27] Dijkstra KK, Monkhorst K, Schipper LJ, et al. Challenges in establishing pure lung cancer organoids limit their utility for personalized medicine. Cell Rep 2020;31(5): 107588.
[28] Gao D, Vela I, Sboner A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell 2014;159(1):176–87.
[29] van de Wetering M, Francies HE, Francis JM, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161(4):933–45.
[30] Clevers H, Loh KM, Nusse R. Stem cell signaling. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. Science 2014;346 (6205):1248012.
[31] Pfister DG, Spencer S, Adelstein D, et al. Head and neck cancers, Version 2.2020, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 2020;18 (7):873–98.