New patient derived head and neck cancer xenograft (PDX) for drug development, immuno-oncology and translational research

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Background and Aim

Head and neck squamous cell carcinomas (HNSCC) represent a heterogeneous group of epithelial cell malignancies arising from the upper aerodigestive tract. Despite of improved therapies, HNSCC remain a devastating disease.
Oropharynx cancers are mainly attributed to infection with human papillomavirus (HPV). Since 2017, HNSCC are classified into HPV-negative and HPV-positive HNSCC. Therapies in the clinic are surgery and/ or radiotherapy (RT)/ radiochemotherapy (RCT) with a recurrence of about 50%. Therefore, new approaches are needed to improve long-term remission and patient survival. Recent advances in high-throughput molecular profiling have helped to identify genetic dispositions (TP53, FAT1, CDKNA2, NOTCH1 etc.) for HNSCC.
We have generated a diverse panel of patient derived xenografts (PDX) of HNSCC for preclinical research and immuno-oncological approaches.

Methods

Our PDX were established from fresh surgery tissue of primary and recurrent tumors or lymph nodes by direct subcutaneous implantation into immune-deficient mice. For characterization, the PDX were treated with standard of cares (SoC) drugs and investigational drugs. Several HNSCC PDX were also treated with RT in comparison to treatment with cetuximab.
In addition, growth of HNSCC PDX on humanized mice was investigated to create new models for the evaluation of novel immunotherapy approaches.
To gain a deeper insight in the molecular biology, RNA sequencing was performed for 46 HNSCC PDX models. In addition to genome-wide gene expression and sequence variation analyses, individual Human Leucocyte Antigen (HLA) profiles comprising HLA class I, II and non-class types in 4-digit resolution were determined.
A comprehensive HLA matching analysis of the HNSCC models and 9 peripheral blood mononuclear cell (PBMC) donors was performed according to donor-recipient HLA matching criteria recommended by the Blood and Marrow Transplant Clinical Trials Network (Howard et al. (2015), DOI: 10.1016/j.bbmt.2014.09.017).

Results

Out of 176 transplanted patient HNSCC, we established and characterized 85 new xenografts (Figures 1-4). 14 PDX were derived from HPV-positive tumors. 28 HNSCC PDX were treated with RT alone or RCT. Heterogeneous individual responses to treatments resemble the clinical situation. For characterization, the PDX were treated with SoC drugs such as docetaxel, platinum compounds, cetuximab, 5 fluorouracil and investigational drugs. Cetuximab response was associated with basal subtype and inflamed/ mesenchymal subtype was negative predictive for cetuximab response (Figures 5 - 7). 30 HNSCC PDX were analyzed for PD-L1 expression (Figure 8) as suitable candidates for the evaluation of checkpoints inhibitors such as nivolumab and other novel immunotherapy approaches on humanized mice. Explorative analyses of RNA sequencing data confirmed the heterogeneity of HNSCC models according to gene expression and sequence variations. Individual HLA profiles were generated comprising HLA-A, -B and -C (class I), HLA-DQA1, -DQB1, -DRB1, -DPA1 and -DPB1 (class II) as well as HLA-H (nonclass) types (Figure 9). HLA profile matching of PDX models and PBMC donors resulted in 6 matches enabling personalized, preclinical immune-oncology studies. The dependence of immunotherapy efficacy on matched HLA profiles was shown exemplarily for HN15239 (Figures 10A-B).

Conclusions

Our comprehensively characterized HNSCC PDX panel enables the evaluation of new targeted and immunological therapies in preclinical phase II studies. It provides an exceptional platform for the identification and validation of new targets and enables the preclinical screening of new combinations in translational research.

Clinical, histological and molecular characteristics of selected HNSCC PDX models

TumorID	TNM	UICC stage	Grading	Age	Site of tumor origin	Gender	Primary/ recurrent	Features
HN9619	T2N0M0	II	NA	NA	Oropharynx	Female	Recurrent	HPV-16 positive
HN9876	T3N2cM0	IVA	G3	62	Hypopharynx	Male	Recurrent	SoC resistent
HN9897	T2N2bM0	IVA	G3	58	Hypopharynx	Male	Recurrent	SoC resistent
HN10110	T2N2cM0	IVA	G2	69	Tongue	Male	Primary	PIK3CA altered
HN10114	T3N0M0	III	G3	52	Oral cavity	Male	Primary	wt
HN10159	T1N0M0	I	G2	57	Oral cavity	Male	Primary	
HN10309	T4N2cM0	IVA	G3	55	Oropharynx	Male	Primary	HPV-16 positive
HN10321	T2N0M0	II	G2	65	Tongue	Male	Primary	SoC resistent
HN10379	T3N2bM0	IVA	G2	39	Soft palate	Male	Primary	
HN10511	T2N0M0	II	G2	54	Oropharynx	Male	Primary	HPV-16 positive
HN10621	T2N2bM0	IVA	G3	61	Oropharynx	Male	Primary	PIK3CA altered
HN10632	T2N1M0	III	G3	60	Tongue	Male	Primary	
HN10847	T2N1M0	III	G2	71	Soft palate	Female	Recurrent	PIK3CA altered
HN10913	T4N2bM0	IVA	G2	50	Oral cavity	Male	Primary	Rezidiv HN13194
HN10924	T3N2cM0	IVA	G2	65	Hypopharynx	Male	Primary	
HN10927	T2N2bM0	IVA	G2	67	Oropharynx	Male	Primary	
HN10960	T2N0M0	II	G2	63	Tongue	Male	Primary	PIK3CA altered
HN10980	T4bN2bM0	IVB	G2	59	Soft palate	Female	Primary	
HN11097	T4aN2bM0	IVA	G2	75	Oral cavity	Female	Primary	PIK3CA altered
HN11142	T2N2cM0	IVA	G3	46	Oral cavity	Male	Primary	
HN11143	T2N2bM0	IVA	NA	82	Oropharynx	Male	Primary	HPV-16 positive
HN11218	T4N0M0	IVA	G2	68	Soft palate	Female	Primary	
HN11269	T4aN2cM0	IVA	G2	71	Oral cavity	Male	Primary	
HN11437	T4bN2cM0	IVB	G2	56	Oral cavity	Male	Primary	
HN11452	T2N0M0	II	G2	75	Oral cavity	Male	Primary	
HN11482	T2N2bM0	IVA	G2	61	Oral cavity	Male	Primary	PIK3CA altered

Figure 1: Clinical characteristics of selected HNSCC PDX.
Reference: K. Klinghammer et al, Int. J. Cancer: 136, 2940-2948 (2015)

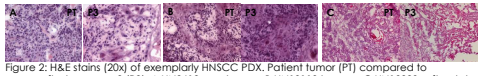

Figure 2: H&E stains (20x) of exemplarly HNSCC PDX. Patient tumor (PT) compared to xenografted passage 3 (P3). A HN9619 oropharynx, B HN10110 tongue, C HN10980 soft palate

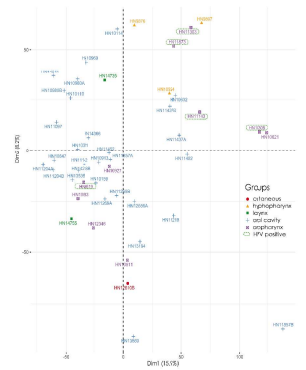

Figure 3: PCA plot shows the variance of gene expression from HNSCC PDX related to their site of tumor origin

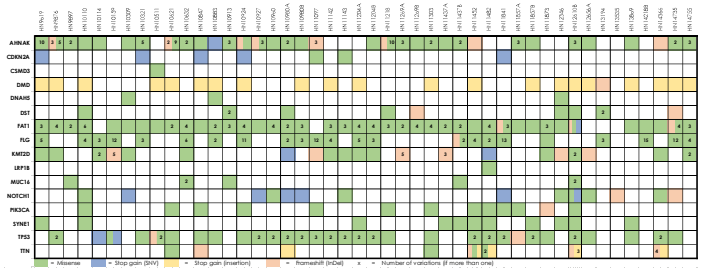

Figure 4: Mutation analysis by means of RNA-Seq of 46 HNSCC PDX models revealed individual profiles with mutations common in HNSCC.

Drug response of selected HNSCC PDX models

PDX ID	HPV-16 status	Drug response according to RECIST*					
		Docetaxel	Cetuximab	Everolimus	Carboplatin	5-FU	Mitoxantrone
HN10110	negative						n/a
HN10114	negative						n/a
HN10621	negative						
HN10632	negative						
HN10980A	negative						
HN10980B	negative						

PDX ID	HPV-16 status	Drug response according to RECIST*					
		Docetaxel	Cetuximab	Everolimus	Carboplatin	5-FU	Mitoxantrone
HN9619	positive						n/a
HN10309	positive						n/a
HN10511	positive						n/a
HN11143	positive						n/a
HN11303	positive				n/a	n/a	n/a
HN11873	positive				n/a	n/a	n/a

* = Progressive disease (PD), = Stable disease (SD), = Partial or complete remission (PR or CR)

Figure 5: Response to SoC´s regarding the stringent clinical response criteria (RECIST) of HPV-negative and HPV-positive HNSCC PDX in immune-deficient mice. The criteria apply the tumor volumes taking into account three dimensions of tumors. The quote of the relative tumor volume (RTV) at the day of evaluation and at start of the treatment is used: RTV of < 0.2 is CR, RTV of < 0.7 is PR, RTV of 0.7-1.3 is SD and RTV > 1.3 is PD.

HPV- and HPV+ HNSCC PDX models in response to SoC´s

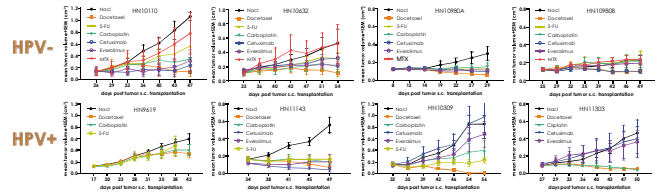

Figure 6: Tumor growth curves from HNSCC PDX in response to SoC´s xenografted in immune-deficient mice. Dose schedule and application route: Docetaxel 12.5 mg/ Kg once weekly x3 iv, Carboplatin 75 mg/ Kg once weekly x3 ip, Cetuximab 50 mg/ Kg once weekly x3 iv, 5 Fluorouracil 100 mg/ Kg once weekly x3 ip, Everolimus 4 mg/ Kg d1–5 x 3 weeks po, Mitoxantrone 10 mg/ Kg every three days x 5 ip; Abbreviations: iv: intravenous, ip: intraperitoneal, po: per os

HNSCC PDX models for radiotherapy and radiochemotherapy research

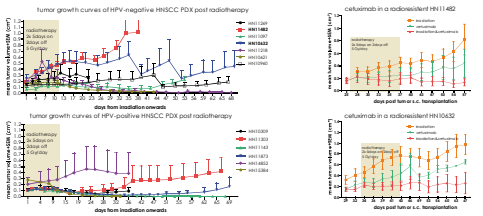

Figure 7: (A) Tumor growth curves from selected HPV- and HPV+ HNSCC PDX after local RT (20 Gy) in immune-deficient mice. HPV+ HNSCC PDX show higher radiosensitivity compared to HPV- HNSCC PDX.
(B) Efficacy of cetuximab in radio-resistent HNSCC PDX.

HNSCC PDX models for immunotherapy research

PD-L1/CD274	HN9619	HN9876	HN9897	HN10110	HN10159	HN10309	HN10621	HN10913	HN10924	HN10960	HN11303	HN11437A	HN11452	HN11482	HN11841	HN11873	HN13194
mRNA	45,14		4,06	2,21	12,11	0,86	28,27	21,70	0,93	0,48	2,58	0,94	1,52	6,05	1,23	1,05	2,08	3,49
protein	73,78	19,46	49,42	8,26	31,03	56,83	52,27	28,9	48	33,32	38,18	68,65	21,47	25,85	47,47	31,03	41,39	

Figure 8: Presence of PD-L1 in selected HNSCC PDX. The relative mRNA expression of PD-L1 contrasted to the protein expression (RNAseq vs FACS). PD-L1 expression is heterogenously distributed and does not correlate between expression of mRNA and protein.

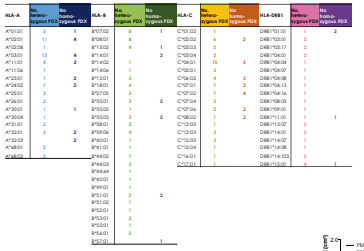

Figure 9: Distribution of HLA-types in 46 HNSCC PDX. The number of PDX represents heterozygous and homozygous occurrences of the respective HLA type (multiple counting possible due to equiprobable alternatives of alleles).

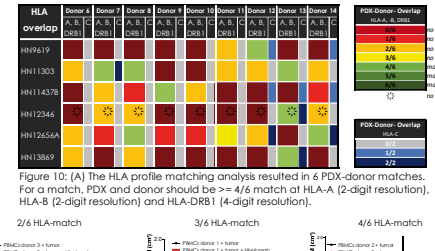

Figure 10: (A) The HLA profile matching analysis resulted in 6 PDX-donor matches. For a match, PDX and donor should be >= 4/6 match at HLA-A (2-digit resolution), HLA-B (2-digit resolution) and HLA-DRB1 (4-digit resolution).

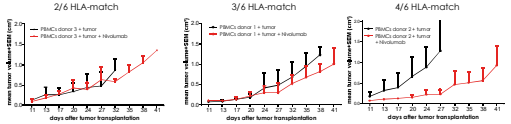

Figure 10: (B) Effect of HLA-matching on efficacy of the checkpoint inhibitor Nivolumab on HNSCC PDX model HN15239 in PBMC-humanized NOG mice. The better is the HLA match the more effective is the treatment with Nivolumab in this HNSCC PDX.

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