First-line treatment of locally advanced HNSCC with double checkpoint blockade and radiotherapy dependent on intratumoral CD8+ T cell infiltration

CheckRad-CD8

Final Report

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Clinical Trials No.: NCT03426657
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Ethikkommission-Bearbeitungsnummer: 131_18 Az
(federführend: Ethikkommission der
Medizinischen Fakultät der Universität Erlangen-Nürnberg)

Investigational drugs	Durvalumab (MEDI4736), Tremelimumab
Sponsor	Universitätsklinikum Erlangen, Strahlenklinik, insoweit handelnd für den Freistaat Bayern, vertreten durch den Dekan der Medizinischen Fakultät der Friedrich-Alexander-Universität Erlangen-Nürnberg
Investigator	Prof. Dr. Rainer Fietkau, Strahlenklinik, Universitätsklinikum Erlangen, Universitätsstr. 27, 91054 Erlangen

1	**Name of Sponsor/Company**
	Universitätsklinikum Erlangen, Strahlenklinik, insoweit handelnd für den Freistaat Bayern, vertreten durch den Dekan der Medizinischen Fakultät der Friedrich-Alexander-Universität Erlangen-Nürnberg
2	**Name of Finished Product**
	Durvalumab (MEDI4736), Tremelimumab
3	**Name of Active Substance**
	Durvalumab (MEDI4736), Tremelimumab, Docetaxel, Cisplatin, Carboplatin
4	**Individual Study Table: Referring to Part of the Dossier (Volume, Page)** **Anmerkung: Diese Angabe ist nur bei Einreichung in Zusammenhang mit einem Zulassungsdossier erforderlich**
	Not applicable
5	**Title of Study** **Anmerkung: Es muss klar hervorgehen, dass die letzte Protokollversion einschließlich aller Amendments gemeint ist, die Amendments sind anzugeben und zu identifizieren**
	First-line treatment of locally advanced HNSCC with double checkpoint blockade and radiotherapy dependent on intratumoral CD8+ T cell infiltration – Protocol v1.7 Previous Protocol Versions/Amendments: • Protocol v. 1.2 – 13.09.202018 First Submission • Protocol v. 1.3 – 20.12.2018 Amendment 1 • Protocol v. 1.4 – 01.04.2020 Amendment 3 • Protocol v. 1.5 – 16.12.2020 Amendment 4 • Protocol v. 1.6 – 01.04.2022 Amendment 5 • Protocol v. 1.7 – 10.02.2023 Amendment 7 End of Recruitment 24.09.2021 End of Trial (last patient last visit): 29.02.2024
6	**Investigators**
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7	**Study centre(s)**	
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	2	Düsseldorf, Universitätsklinikum
	3	Frankfurt, Universitätsklinikum
	4	Regensburg, Universitätsklinikum
	5	Dresden, BAG/Onkologische Gemeinschaftspraxis
	6	Otto von Guericke Universität Magdeburg
	7	Ulm, Universitätsklinikum
	8	Klinikum Chemnitz gGmbH
8	**Publication (reference)**	

Semrau S, Schmidt D, Hecht M, et al. Classification of three prognostically different groups of head and neck cancer patients based on their metabolic response to induction chemotherapy (IC-1). Oral Oncol. 2020;100:104479.

Hecht M, Gostian AO, Eckstein M, et al. Safety and efficacy of single cycle induction treatment with cisplatin/docetaxel/ durvalumab/tremelimumab in locally advanced HNSCC: first results of CheckRad-CD8. J Immunother Cancer. 2020;8(2):e001378.

Semrau S, Gostian AO, Traxdorf M, et al. Implementation of Double Immune Checkpoint Blockade Increases Response Rate to Induction Chemotherapy in Head and Neck Cancer. Cancers (Basel). 2021;13(8):1959. Published 2021 Apr 19.

Hellwig K, Ellmann S, Eckstein M, et al. Predictive Value of Multiparametric MRI for Response to Single-Cycle Induction Chemo-Immunotherapy in Locally Advanced Head and Neck Squamous Cell Carcinoma. Front Oncol. 2021;11:734872. Published 2021 Oct 21.

Weissmann T, Speer S, Putz F, et al. Reduction of Elective Radiotherapy Treatment Volume in Definitive Treatment of Locally Advanced Head and Neck Cancer-Comparison of a Prospective Trial with a Revised Simulated Contouring Approach. J Clin Med. 2021;10(20):4653. Published 2021 Oct 11.

Beck M, Semrau S, Haderlein M, et al. Differences and Similarities in the Pattern of Early Metabolic and Morphologic Response after Induction Chemo-Immunotherapy versus Induction Chemotherapy Alone in Locally Advanced Squamous Cell Head and Neck Cancer. Cancers (Basel). 2022;14(19):4811. Published 2022 Sep 30.

Hecht M, von der Grün J, Semrau S, et al. Highlights der ASCO- und ESMO-Jahrestagungen 2021 zur Strahlentherapie von Kopf-Hals-Tumoren [Highlights from the 2021 ASCO and ESMO annual meetings on radiotherapy of head and neck cancer]. HNO. 2022;70(4):258-264.

	Hecht M, Eckstein M, Rutzner S, et al. Induction chemoimmunotherapy followed by CD8+ immune cell-based patient selection for chemotherapy-free radioimmunotherapy in locally advanced head and neck cancer. J Immunother Cancer. 2022;10(1):e003747. *Beck M, Hartwich J, Eckstein M, et al. F18-FDG PET/CT imaging early predicts pathologic complete response to induction chemoimmunotherapy of locally advanced head and neck cancer: preliminary single-center analysis of the checkrad-cd8 trial. Ann Nucl Med. 2022;36(7):623-633.* *Hecht M, Frey B, Gaipl US, et al. Machine Learning-assisted immunophenotyping of peripheral blood identifies innate immune cells as best predictor of response to induction chemo-immunotherapy in head and neck squamous cell carcinoma - knowledge obtained from the CheckRad-CD8 trial. Neoplasia. 2024;49:100953.*
9	**Studied period (years): date of first enrolment, date of last completed** **Anmerkung: Hier sollen auch Studienunterbrechungen und vorzeitige Studienbeendigungen/Studienabbrüche unter Angabe der Gründe aufgeführt werden**
	First patient in: 20.09.2018 Last patient out of therapy: 20.01.2022 End of Follow up: 29.02.2024
10	**Phase of development**
	Phase II trial
11	**Objective**
	Primary Objective: Assessment of the feasibility of a new treatment scheme with induction Chemo-Immuno-Therapy followed by Radio-Immuno-Therapy Feasibility criteria are receiving the protocol treatment until cycle 6 of antibody treatment and absence of any of the dose limiting toxicities defined in the protocol. A feasibility rate of ≥80% is expected. Assessment of the predictive character of changes of CD8+ tumor infiltrating immune cells after induction chemo-immunotherapy **Secondary Objective:** Assessment of the efficacy of a Radio-Immuno-Therapy with durvalumab and tremelimumab **Exploratory Objectives:** Assessment of predictive value and changes of different tumor infiltrating immune cells and immunological tumor markers. Longitudinal analysis of the immune phenotype in the peripheral blood Toxicity with enhanced or without CTLA-4 blockade (“Expansion Cohort 1 and 2”)
12	**Methodology**
	CheckRad-CD8 is a single-arm multicentre phase II study. Patients with stage III–IVB head and neck squamous cell carcinoma were eligible for this multicentre phase II trial. Treatment consisted of a single cycle of cisplatin 30 mg/m² days 1–3, docetaxel 75 mg/m² day 1, durvalumab 1500 mg fix dose day 5 and tremelimumab 75 mg fix dose day 5. Patients with

	increased intratumoral CD8 +immune cell density or pathological complete response (pCR) in the rebiopsy entered RIT up to a total dose of 70 Gy. Patients received further three cycles of durvalumab/tremelimumab followed by eight cycles of durvalumab mono (every 4 weeks). The intended treatment for patients not meeting these criteria was standard radiochemotherapy outside the trial. Primary endpoint was a feasibility rate of patients entering RIT to receive treatment until at least cycle 6 of immunotherapy of ≥80%.
13	**Number of patients (planned and analysed)**
	Planned: • Enrolment: 121 (one patient has withdrawn consent before start of therapy) Analysed: • Main cohort: 80 • Expansion cohort 1: 20 • Expansion cohort 2: 20
14	**Diagnosis and main criteria for inclusion**
	Diagnosis: Histologically confirmed, locally advanced unresectable pancreatic cancer UICC III, no distant metastasis present **Main criteria for inclusion:** Locally advanced HNSCC, UICC stage III-IVB (oral cavity, oropharynx, hypopharynx, supraglottic larynx) (according to TNM version 8) Histological confirmation of HNSCC (regardless if p16 positive or negative) Measurable CD8 density in provided archival tumor tissue ECOG performance status ≤1 Age ≥ 18 Written informed consent for the participation in the clinical trial
15	**Test product, dose and mode of administration, batch number**
	Durvalumab is a human monoclonal antibody (mAb) of the immunoglobulin G1 kappa subclass that blocks the interaction of PD-L1 (but not PD-L2) with PD-1 on T cells and CD80 on immune cells. In vitro studies demonstrate that durvalumab antagonizes the inhibitory effect of PD-L1 on primary human T cells, resulting in their restored proliferation and release of interferon gamma.3 **Tremelimumab** is a human immunoglobulin G2 mAb that is directed against cytotoxic T-lymphocyte associated antigen 4 (CTLA-4; CD152), a cell surface receptor that is expressed primarily on activated T cells and acts to inhibit their activation. Tremelimumab completely blocks the interaction of human CTLA-4 with CD80 and CD86, resulting in increased release of cytokines interleukin-2 and interferon gamma from human T cells, peripheral blood mononuclear cells and whole blood4, thus enhancing T-cell activation. Durvalumab and tremelimumab are supplied by AstraZeneca as vial solutions for infusion after dilution.

Dose and mode of administration

All patients will initially be treated with the PD-L1 inhibitor Durvalumab and the CTLA-4 Inhibitor Tremelimumab ("Main cohort" 75mg, "Expansion cohort 1" 300mg, "Expansion cohort 2" no Tremelimumab) and one cycle with Cisplatin (30mg/m² d1-3) and Docetaxel (75mg/m² d1).

Treatment response will be evaluated clinically by endoscopy with biopsy. Changes of the CD8+ T cell density in the second biopsy compared to the first one before therapy will be used for patient selection.

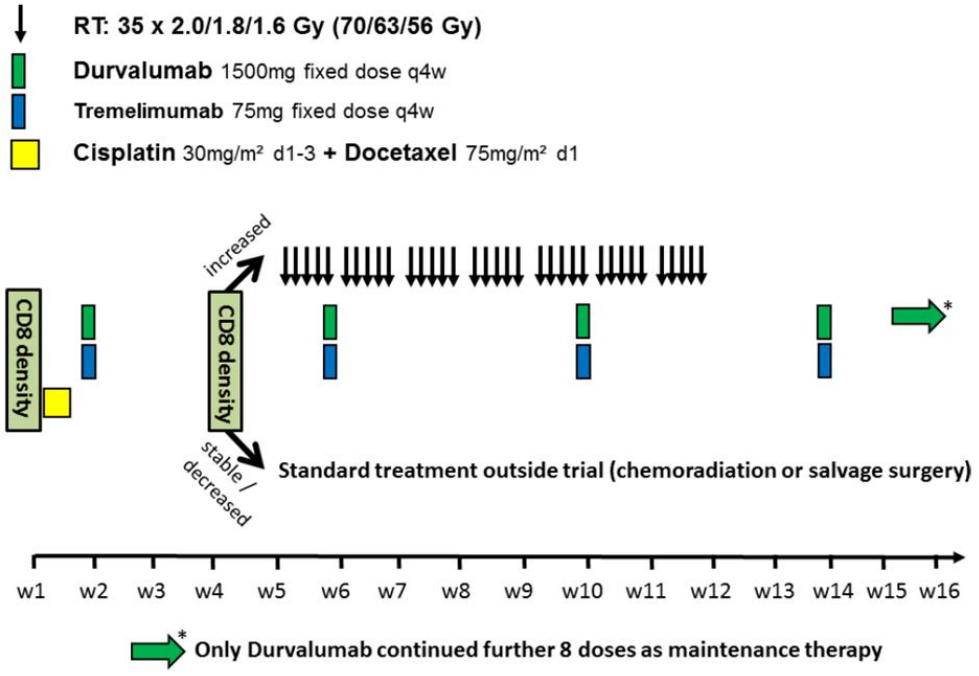
Patients with a stable or decreased CD8+ tumor infiltrating immune cell density or clinical progressive disease will receive standard CRT outside the trial. For these patients, toxicity will be monitored until 90 days after last administration of durvalumab and/or tremelimumab or until the first dose of the subsequent standard CRT whichever occurs first.

Patients with an increased CD8+ tumor infiltrating immune cell density and at least clinically stable disease will receive radioimmunotherapy with the PD-L1 Inhibitor durvalumab and the CTLA4-inhibitor tremelimumab followed by maintenance therapy with durvalumab (8 additional doses q4w).

Radiotherapy will be delivered to the local tumor with linear accelerators as intensity modulated radiation therapy of volumetric modulated arc therapy.

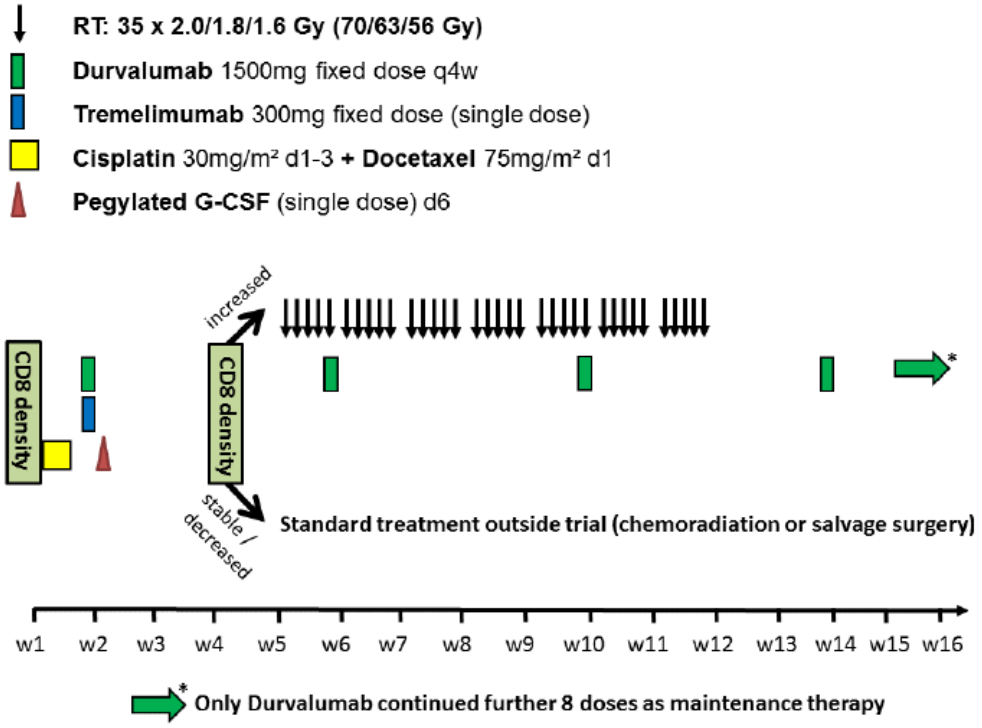
Main cohort:

Durvalumab 1500 mg plus tremelimumab 75 mg via IV infusion q4W for up to a maximum of 4 doses, followed by durvalumab monotherapy 1500mg via IV infusion q4W, starting 4 weeks after the infusion of the combination, for up to a maximum of 8 additional durvalumab doses.



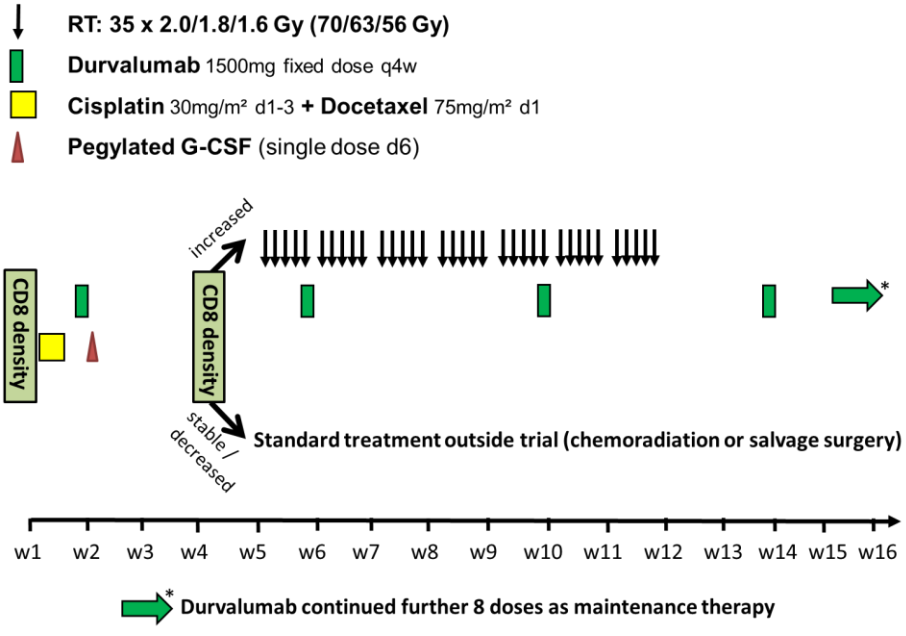
Expansion cohort 1

Durvalumab 1500 mg plus tremelimumab 300 mg via IV infusion followed by durvalumab monotherapy 1500mg via IV infusion q4W, starting 4 weeks after the infusion of the combination, for up to a maximum of 11 additional durvalumab doses.



Expansion cohort 2

Durvalumab monotherapy 1500 mg via IV infusion q4W for up to a maximum of 12 durvalumab doses.



d = day; Gy = Gray; q4w = every four weeks; RT = radiotherapy; w = week

16	**Duration of treatment**
	46 weeks
17	**Reference therapy, dose and mode of administration, batch number**
	Not applicable
18	**Criteria for evaluation Efficacy, Safety**
	Safety Assessments: Toxicity will be documented according to CTCAE v4.03 before every administration of durvalumab/tremelimumab. An interim analysis will be performed after the first 20 patients have completed the radioimmunotherapy. **Efficacy Assessments:** Progression-free survival, overall survival and pathological confirmed response rate
19	**Statistical methods**
	Sample Size Calculation **Main cohort:** Conventional empirical phase I study designs in clinical oncology assume, that an antineoplastic treatment is not feasible, if an unacceptable toxicity occurs in more than 1 out of 3 or 4 patients; however, the occurrence of dose limiting toxicities (DLT) in 1/6 is accepted. This leads to the conclusion that the limit of acceptance is considered to be around 20%. A one-stage design for pilot studies according to Fleming (1982) is applied. In summary, the trial design is based on the following assumptions: • The experimental therapy would be rated as unacceptable, if the actual feasibility rate (= 1 – rate with DLT events) was only 65% or lower. • On the other hand, the therapy regimen would be considered to be a promising candidate for further development, if the true feasibility rate amounted to 80% or more. • Probability to accept the experimental therapy as well tolerable, in spite of a true feasibility rate of < 65% (i.e., rate with DLT > 35%): 5% (type I error) • Probability to reject the experimental therapy as not sufficiently feasible (<65%), although the true feasibility rate is promising (> 80%): 20% (type II error, corresponding to a power of 80%). According to these parameters n = 56 patients evaluable for feasibility have to be recruited into the trial. In order to allow for about 50% with insufficient immune response, plus some non-informative drop-outs, a total number of 120 patients should be recruited. Recruitment is completed as soon as n= 56 patients entered radioimmunotherapy. **Expansion cohort 1 and 2:** The toxicity of two additional immune-oncologic treatment schemes with enhanced CTLA-4 blockade (Tremelimumab 300 mg Cx1) and without CTLA-4 blockade will be studied. For

these toxicity analyses n= 20 patients should be recruited sequentially for each treatment scheme.

Results

Main cohort

Patient characteristics

Eighty patients were enrolled from September 2018 to May 2020 in eight German centers. Data cut-off was June 7, 2021. One patient did not receive any study treatment due to tumor bleeding and was excluded from all analyses. Baseline characteristics are given in table 1.

During the trial the TNM seventh edition was replaced by the eighth edition. Consequently, all tumor stages were adapted to the eighth edition. In addition, the UICC stages according to the TNM seventh edition are given in online supplemental table 1. PD-L1 status of tumor and immune cells was scored as percentage of PD-L1 positive area of total tumor or immune cell area, respectively, with the previously established cut-off value of 25% for durvalumab ±tremelimumab in HNSCC.

Treatment parameters and feasibility analysis

Seventy-nine patients received induction chemo-immunotherapy (figure 1). Seven patients (9%) received carboplatin instead of cisplatin. Two patients developed relevant toxicity after induction chemotherapy and consequently received no immune checkpoint inhibitors. Restaging assessment including rebiopsy of the primary tumor was performed in 76 of 79 treated patients. Pathologic response was pCR in 41 patients (52%, 95% CI 37% to 60%). Of the remaining 35 patients, 31 (39%) had an intratumoral increase of CD8 +immune cells, with a median increase by factor 3.0. Taken together, 72 patients fulfilled the criteria to continue trial treatment. Out of these, seven patients had to be excluded due to toxicity, mainly elevated transaminases/hepatitis (n=4), and five patients opted for alternative treatments. Taken together, 19 patients treated with induction chemo-immunotherapy did not enter RIT and mostly received radiotherapy combination treatments in a curative intent (17 patients). Thus, 60 patients (76%) entered RIT representing the primary endpoint cohort, which fulfills the predefined calculated sample size of n=57 subjects to analyze the primary endpoint feasibility until cycle 6 of immunotherapy. Radiotherapy to a cumulative dose of at least 66.0/59.4/50.8 Gy (at least 33 of planned 35 fractions) was delivered in 59 patients. One patient terminated radiotherapy prematurely without toxicity on her own request (no DLT). Immunotherapy was terminated before cycle six in three additional patients for other reasons than DLT, which were excluded from the feasibility analyses according to the study protocol. Ten patients experienced DLT, namely three cases of elevated transaminase / hepatitis, two cases of arthritis, one nephritis, one pancreatitis, one adrenalitis, one pneumonitis and one hypothyroidism. Forty-six patients received immunotherapy until at least cycle 6. This results in a feasibility rate of 82% (46/56), meeting the primary endpoint of the study, as the lower boundary of the one-sided 95% CI is 72%, thus excluding the pre-defined level of unacceptable feasibility (≤65%). In addition to this protocol-specified feasibility analysis, an additional analysis of the entire treatment scheme was performed. In the entire treatment scheme consisting of induction chemo-immunotherapy and RIT 46 of 79 patients (58%) completed cycle six of immunotherapy, whereas after exclusion of ten non-toxicity-related drop outs the overall feasibility rate was 67% (46/69).

Table 1 Patient characteristics of treated patients

	No (n=79)	%
Age (median, SD)	60.2±8.6 years	
Sex		
Male	65	(82)
Female	14	(18)
ECOG performance status		
0	62	(78)
1	17	(22)
Primary tumor site		
Oral cavity	10	(13)
Oropharynx	43	(54)
Hypopharynx	14	(18)
Larynx	12	(15)
T category		
T1	5	(6)
T2	12	(15)
T3	17	(22)
T4	45	(57)
N category		
N0	20	(25)
N1	18	(23)
N2	29	(37)
N3	12	(15)
UICC stage (according to TNM eighth edition)		
II*	5	(6)
III	30	(38)
IV	44	(56)
Tobacco smoking status		
Current smoker	33	(42)
Former smoker	33	(42)
Never smoker	13	(16)
Pack years of current/former smokers (median, SD)	40.0±18.3 pack years	
Intratumoral CD8 +immune cells (IC) (median, range)	391 cells/mm² (12–5984)	
PD-L1 status		
Tumor cells		
<25%	61	(77)
≥25%	18	(23)
IC area		
<25%	49	(62)
≥25%	30	(38)
Algorithm positivity		
Negative	40	(51)
Positive	39	(49)
HPV status all tumors (p16 positivity)		
Negative	53	(67)
Positive	26	(33)
HPV status Oropharynx only (p16 positivity) (n=43)		
Negative	20	(47)
Positive	23	(53)

*The TNM version changed from the seventh edition to the eighth edition during the trial. TNM classification in this table is according to the eighth edition. TNM classification according to the seventh edition is given in online supplemental table 1. HPV, human papilloma virus; PD-L1, programmed death protein ligand 1; TNM, TNM Classification of Malignant Tumors.

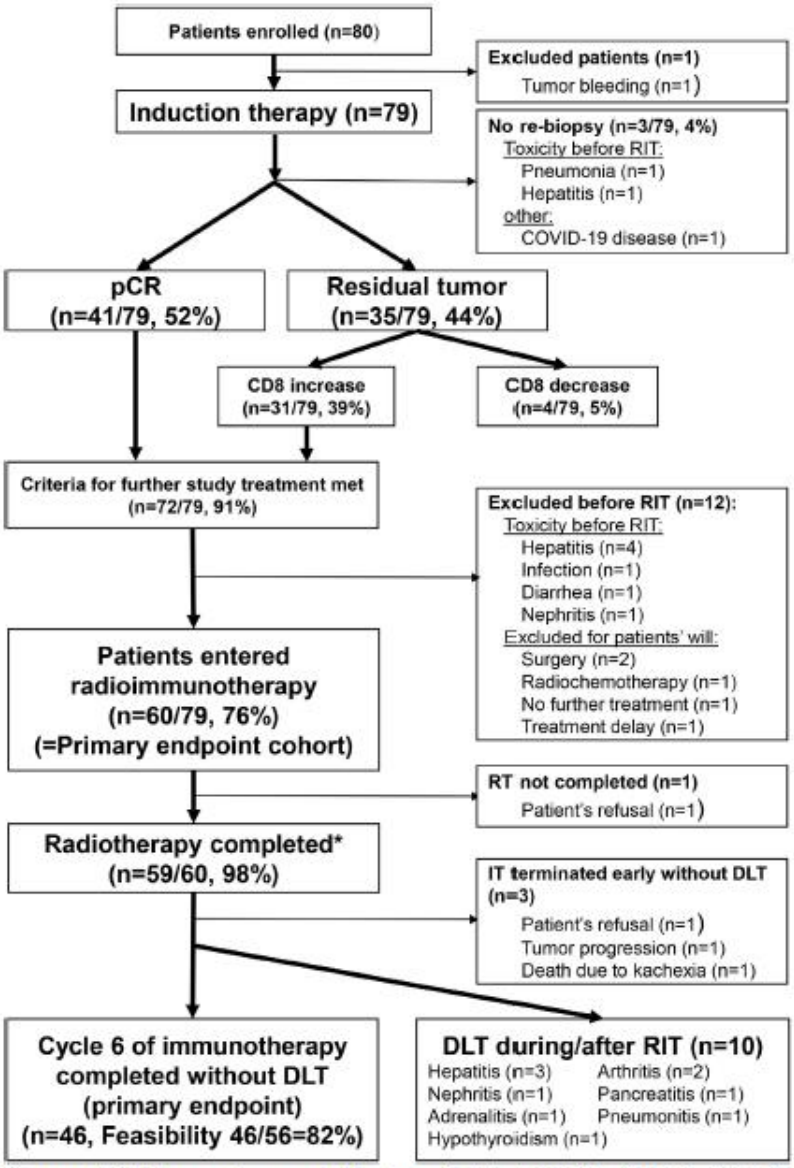


Figure 1 CONSORT diagram. *Radiotherapy to a cumulative dose of at least 66.0/59.4/50.8 Gy. CD8, CD8 +intratumoral immune cells. CONSORT, Consolidated Standards of Reporting Trials; DLT, dose-limiting toxicity; IT, immunotherapy; pCR, pathologic complete response; RIT, radioimmunotherapy; RT, radiotherapy.

Survival analyses

Key secondary endpoints were PFS and OS. The median follow-up was 17.2 months. All patients on treatment completed the protocol-defined restaging assessment 12 weeks after RIT, which was mainly performed with 18F-FDG PET/CT. In the RIT cohort (primary endpoint cohort, n=60) 17 PFS events (28%) and ten deaths (17%) were observed. In the entire study cohort 25 PFS events (32%) and 16 deaths (20%) were detected. Locoregional progression, distant metastases or both appeared in eight, two and one patients in the RIT cohort and nine, four and two patients in the entire cohort, respectively. Three patients receiving neck dissection ≤20 weeks after completion of radiotherapy with pathological detection of residual tumor were not classified as PFS events. and 72%, respectively (figure 2A), and the 1-year and 2-year OS rate was 90% and 84%, respectively (figure 2B). Patients who did not continue with RIT after induction treatment had a 1-year and 2-year PFS rate of 63% and 58% and a 1-year and 2-year OS rate of 79% and 67%. In the entire cohort the 1-year and 2-year PFS rate was 75% and 68%, respectively (figure 2C), and the 1-year and 2-year OS rate was 87% and 79%, respectively (figure 2D). An explorative subgroup analysis of HPV-associated p16

positive oropharyngeal cancers (n=23) detected one case of distant metastases in the RIT cohort and one death in the patients with alternative treatment. There was no locoregional failure in this subgroup. The 2-year PFS rate was 94% in the RIT cohort and 91% in the entire cohort. Patients with other than p16 positive oropharyngeal tumors achieved a 2-year PFS rate of 64% in the RIT cohort and 59% in the entire cohort. In both cohorts, PFS and OS were significantly longer in p16 positive oropharyngeal tumors.

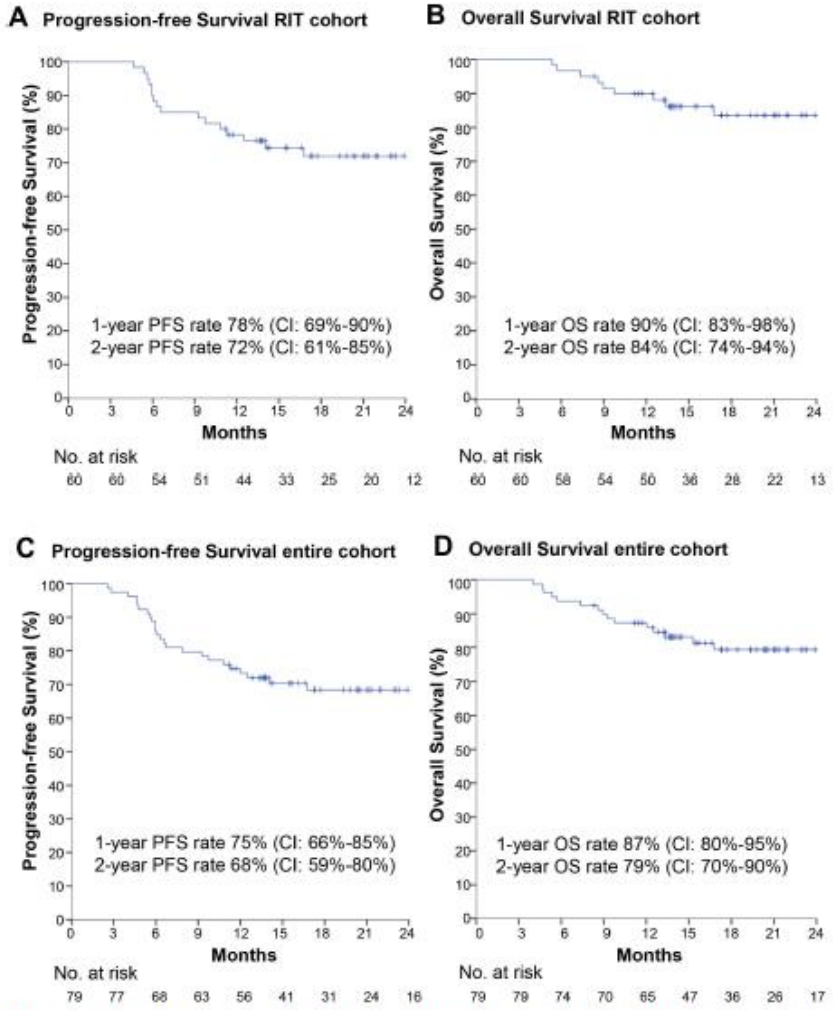


Figure 2 Kaplan-Meier estimates of progression-free (PFS) and overall survival (OS). Kaplan-Meier estimates of (A) PFS and (B) OS of the radioimmunotherapy (RIT) cohort. Kaplan-Meier estimates of (C) PFS and (D) OS of the entire study cohort. Tick marks indicate censored observations.

Safety analyses

Adverse events (AEs) of any cause (treatment related or unrelated) occurring in at least 5% of treated patients are listed in table 2. In addition, AE possibly related to immunotherapy (immune-related AEs, irAE) occurring in any patient are listed. Among all patients treated, 73 patients (92%) experienced a grade 3 AE and 14 patients (18%) experienced a grade 4 AE, with 74 patients (94%) having at least one grade 3–4 AE. There appeared no grade 5 AE. Most common grade 3–4 AE were typical chemotherapy-related events as leukopenia (52%) and infections (32%). Further very common AEs grade 3–4 were typical radiotherapy-related, such as dysphagia (53%), stomatitis (14%) and radiation dermatitis (9%). AE grade 3–4 possibly related to immunotherapy occurred in 23 patients (29%) and mainly included elevated transaminases/hepatitis in eight patients (10%) and diarrhea/colitis in five patients (6%). Out of these, two patients recovered from elevated transaminases/ hepatitis without additional treatment (one had no prior immune checkpoint inhibitor treatment). The other six

patients with elevated transaminases/hepatitis received glucocorticoids, one in combination with mycophenolat-mofetil. All patients recovered, whereas one developed a primary sclerosing cholangitis as possible late complication. Endocrinopathies of any grade included hyperthyroidism (18%) that resulted in subsequent hypothyroidism (19%) in most cases. Endocrinopathies grade 3 included adrenalitis and hypophysitis with two cases each that were treated with hydrocortisone replacement. Two patients developed COVID-19 disease during study treatment. Both patients had only mild symptoms, but one patient was withdrawn from the study due to delayed restaging assessments.

Biomarker analyses

Treatment failure defined as locoregional tumor recurrence, residual locoregional disease or distant metastases (RRM) served as endpoint for the explorative biomarker analysis in the RIT cohort. A total of 55 patients treated with RIT provided full availability of pathological and liquid immune parameters (out of 60 patients, 92%) and were included in this analysis. Intratumoral CD8 +immune cell density and PD-L1 on tumor cells were not associated with treatment failure (figure 3A, B). Low PD-L1 immune cell area predicted treatment failure (RRM; p=0.0419, figure 3C). In the analysis of liquid immune parameters, all analyzed 54 immune cell subsets were normalized for these three parameters and additionally for T-stage and HPV/p16 status. The rare immune cell subsets human leukocyte antigen – DR isotype (HLA-DR) expressing B cells, dendritic cells (DC) and their subgroup myeloid DCs (mDC) and double negative T cells (DNT) were significantly associated and further 11 immune cell subsets mainly from the innate compartment tended to be associated ($p<0.2$) with RRM (figure 3D–G, online supplemental table 3). The slight, but significant differences of immune cell subsets in the peripheral blood when comparing RRM with non-RRM are in the expected range for serving as immune biomarkers in cancer disease.

Table 2 Adverse events (AE) of treated patients

	Grade 1–2		Grade 3		Grade 4	
n=79 patients	**No**	**%**	**No**	**%**	**No**	**%**
Non immune-relate AE appearing in ≥5% of patients (non-irAE)						
Alopecia	64	(81)	0		0	
Fatigue	52	(66)	8	(10)	0	
Xerostomia	55	(70)	4	(5)	0	
Dysphagia	15	(19)	42	(53)	0	
Infection	29	(37)	23	(29)	2	(3)
Leukopenia	13	(16)	31	(39)	10	(13)
Stomatitis	40	(51)	11	(14)	0	
Radiation dermatitis	42	(53)	7	(9)	0	
Nausea	41	(52)	3	(4)	0	
Pain	38	(48)	5	(6)	0	
Pruritus	37	(47)	0		0	
Constipation	32	(41)	0		0	
Vertigo	30	(38)	1	(1)	0	
Lymph edema	30	(38)	0		0	
Vomiting	25	(32)	1	(1)	0	
Oral thrush	25	(32)	0		0	
Polyneuropathy	23	(29)	0		0	
Thrombocytopenia	20	(25)	0		0	
Hypokalemia	13	(16)	3	(4)	0	
Dyspnea	15	(19)	0		0	
PEG/catheter complication	10	(13)	5	(6)	0	
Hoarseness	14	(18)	0		0	
Renal insufficiency	10	(13)	3	(4)	1	(1)
Subcutaneous fibrosis	13	(16)	0		0	
Hyperpigmentation	12	(15)	0		0	
Anemia	8	(10)	3	(4)	0	
Hearing disorder	11	(14)	0		0	
Ear disorder	9	(11)	1	(1)	0	
Hyponatremia	4	(5)	4	(5)	0	
Bleeding	5	(6)	1	(1)	1	(1)
Edema	6	(8)	1	(1)	0	
Dysgenusia	4	(5)	1	(1)	0	
Weight loss	3	(4)	2	(3)	0	
Ulcus (tumor location)	3	(4)	0		1	(1)
Trism	4	(5)	0		0	
Any possibly irAE						
Diarrhea/colitis	40	(51)	5	(6)	0	
Skin reaction	27	(34)	0		0	
Elevated transaminases/ hepatitis	7	(9)	6	(8)	2	(3)
Hypothyroidism	14	(18)	1	(1)	0	
Hyperthyroidism	14	(18)	0		0	
Arthritis	4	(5)	1	(1)	0	
Increased lipase/pancreatitis	0		3	(4)	0	
Adrenalitis	0		2	(3)	0	
Hypophysitis	0		2	(3)	0	
Nephritis	1	(1)	1	(1)	0	
Pneumonitis	0		0		1	(1)

Non-irAE appearing in at least 5% of patients independent from relationship to treatment and all possibly irAE.
PEG, percutaneous endoscopic gastrostomy.

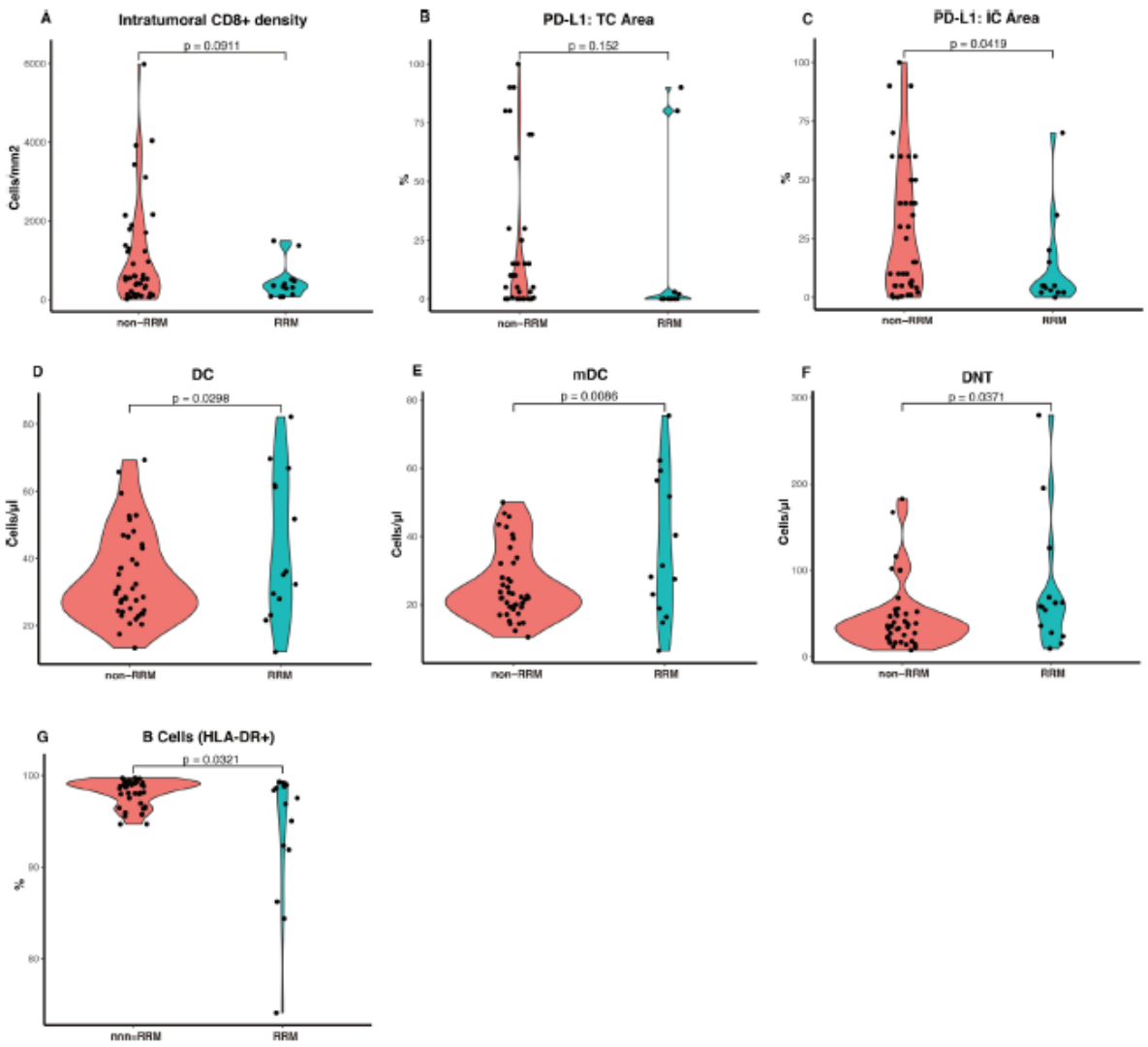


Figure 3 Predictive immune parameters of treatment failure. Comparison of the histological parameters (A) intratumoral CD8 +cell density as determined by immunohistochemistry, (B) programmed cell death ligand 1 (PD-L1) tumor cell area (TC area), (C) PD-L1 immune cell area (IC area) and the liquid immune parameters (D) dendritic cells (DC; LIN-/HLA-DR+), (E) myeloid DCs (mDC; LIN-/HLA-DR+/CD11c high, CD1c-, CD123 low), (F) double negative T cells (DNT; CD3+/CD4-/CD8-), and (G) HLA-DR +B cells (CD19+/CD20+) in patients with locoregional tumor recurrence, residual locoregional disease or distant metastases (RRM) and without RRM (non-RRM). HLAD-DR, human leukocyte antigen – DR isotype.

Expansion cohort 1 and 2

In group EC1, the feasibility rate is 78.6%. The one-sided 90% confidence interval ranges from 56.8% to 91.1%, while the 95% confidence interval lies between 52.4% and 92.4%. For group EC2, the feasibility rate is 82.4%, with a one-sided 90% confidence interval of 63.1% to 92.7% and a 95% confidence interval ranging from 59.0% to 93.8%.

In addition to 80 patients enrolled in the main cohort (MC, one excluded), another 20 patients were enrolled in extension cohort 1 (EC1) and another 20 in extension cohort 2 (EC2, one excluded). In the MC, EC1 and EC2 a total of 56%, 50%, 58% were stage IV and 29%, 30%, 26% had p16 positive oropharyngeal tumors. Baseline median intratumoral CD8+ immune cell density was 395/mm², 505/mm² and 763/mm² in MC, EC1 and EC2. After induction chemo-immunotherapy 41 (52%), 12 (60%) and 11 (58%) of the patients had pathological complete response (pCR) in the re-biopsy in MC, EC1 and EC2. Patients with residual tumor after induction therapy had a median intratumoral CD8+ immune cell density of 670/mm², 781/mm² and 1605/mm², which was a median increase by factor 3.0, 2.1

and 4.8 in the corresponding patients' tissue samples. In the cohorts MC, EC1 and EC2 the overall rate of grade 3-4 adverse events per patient was 1.38, 1.35 and 0.58. The corresponding rate of non-hematologic adverse events per patient was 0.84, 0.95 and 0.37, respectively (see table 3).

Table 3: Comparison of cohorts regarding pathological outcome and toxicities grade 3 & 4

	Main cohort	**Expansion cohort 1**	**Expansion cohort 2**
Enrolled patients (n)	80	20	20
Excluded patients (n)	1	0	1
Proportion of locally advanced HNSCC stage IV (%) (%)	56	50	58
p16+ tumors (%)	29	30	26
Baseline median intratumoral CD8+ density (/mm2)	395	505	763
Median intratumoral CD8+ density after induction therapy (/mm2)	670	781	1605
Median increase factor	3.0	2.1	4.8
pathological complete response (pCR) after induction therapy n, (%)	41 (52)	12 (60)	11 (58)
Overall grade 3-4 adverse events, per patient	1.38	1.35	0.58
Non-hematologic grade 3-4 adverse events, per patient	0.84	0.95	0.37

Overall Feasibility

The overall analysis included patients from both the main cohort and the expansion cohorts. Initially, data from the main cohort were considered, which comprised 56 patients undergoing RIT, of whom 10 patients developed a DLT. Additionally, 4 patients discontinued therapy for other reasons. Data from the expansion cohorts were subsequently integrated, encompassing 31 patients – 14 patients in group EC1 and 17 patients in group EC2. Across these cohorts, 4 DLTs were observed (2 in EC1, 2 in EC2).

In total, the analysis includes 87 patients, of whom 14 patients developed a DLT. The remaining patients successfully completed the therapy without a DLT. This results in an overall feasibility rate of 83.9%. The one-sided confidence intervals indicate that the overall feasibility lies between 76.4% and 89.3% with 90% confidence, and between 74.8% and 90.2% with 95% confidence.

20	**Summary – Conclusions**
	Main cohort Between September 2018 and May 2020, 80 patients were enrolled (one excluded). Out of these, 23 patients had human papilloma virus (HPV)-positive oropharyngeal cancer. Median follow-up was 17.2 months. After induction chemoimmunotherapy 41 patients had pCR and 31 had increased intratumoral CD8 +immune cells. Of 60 patients entering RIT (primary endpoint cohort), 10 experienced imiting toxic (mainly hepatitis) and four discontinued for other reasons, resulting in a feasibility rate of 82%. The RIT cohort (n=60) had a progression-free survival (PFS) rate at one and 2 years of 78% and 72%, respectively, and an overall survival rate at one and 2 years of 90% and 84%, respectively. Patients with HPV-positive oropharyngeal cancers had greater benefit from RIT with a 2-year PFS rate of 94% compared with 64% for HPV-negative oropharyngeal cancers and other locations. In the entire study cohort (n=79) the 2-year PFS rate was 68% (91% for HPV-positive oropharynx vs 59% for others). Toxicity grade 3–4 mainly consisted of dysphagia (53%), leukopenia (52%) and infections (32%). **Expansion cohort 1 and 2** The trial met the primary endpoint feasibilities of RIT for both expansion cohorts. In group EC1, the feasibility rate is 78.6%, for group EC2, the feasibility rate is 82.4%. Both expansion cohorts showed that neither increase of tremelimumab dosage nor its omission did significantly affect pathologic response to induction chemo-immunotherapy with cisplatin/ docetaxel/ durvalumab. Non-hematologic toxicity was slightly increased for high dose tremelimumab and clearly decreased without tremelimumab. **Overall Feasibility** In total, the analysis includes 87 patients, of whom 14 patients developed a DLT. The remaining patients successfully completed the therapy without a DLT. This results in an overall feasibility rate of 83.9%. The one-sided confidence intervals indicate that the overall feasibility lies between 76.4% and 89.3% with 90% confidence, and between 74.8% and 90.2% with 95% confidence. **Conclusions** The trial met the primary endpoint feasibility of RIT. Induction chemo-immunotherapy followed by chemotherapy-free RIT after intratumoral CD8 +immune cell-based patient selection has promising PFS.
21	**Date of report**
	20.12.2024

Erlangen, 20.12.2024
Date

Prof. Dr. med. Rainer Fietkau