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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

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ABSTRACT

Purpose: Individual prediction of treatment response is crucial for personalized treatment in multimodal approaches against head-and-neck squamous cell carcinoma (HNSCC). So far, no reliable predictive parameters for treatment schemes containing immunotherapy have been identified. This study aims to predict treatment response to induction chemo-immunotherapy based on the peripheral blood immune status in patients with locally advanced HNSCC.

Methods: The peripheral blood immune phenotype was assessed in whole blood samples in patients treated in the phase II CheckRad-CD8 trial as part of the pre-planned translational research program. Blood samples were analyzed by multicolor flow cytometry before (T1) and after (T2) induction chemo-immunotherapy with cisplatin/docetaxel/durvalumab/tremelimumab. Machine Learning techniques were used to predict pathological complete response (pCR) after induction therapy.

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Results: The tested classifier methods (LDA, SVM, LR, RF, DT, and XGBoost) allowed a distinct prediction of pCR. Highest accuracy was achieved with a low number of features represented as principal components. Immune parameters obtained from the absolute difference (|T2-T1|) allowed the best prediction of pCR. In general, less than 30 parameters and at most 10 principal components were needed for highly accurate predictions. Across several datasets, cells of the innate immune system such as polymorphonuclear cells, monocytes, and plasmacytoid dendritic cells are most prominent.
Conclusions: Our analyses imply that alterations of the innate immune cell distribution in the peripheral blood following induction chemo-immuno-therapy is highly predictive for pCR in HNSCC.

Introduction

Immune checkpoint inhibitors that inhibit the programmed death protein 1 (PD-1)/programmed death protein ligand 1 (PD-L1) pathway are efficient in the treatment of recurrent and/or metastatic head and neck squamous cell carcinoma (HNSCC) [1,2]. In surgically not resectable locally advanced HNSCC, definitive radiochemotherapy is the treatment of choice [3]. Another treatment strategy especially for laryngeal and hypopharyngeal cancer is the administration of induction chemotherapy followed by radio(chemo)therapy [4,5]. Whereas induction chemotherapy failed to improve survival compared to radiochemotherapy alone [6], it can be used to identify patients with biologically sensitive tumors that can successfully be treated with larynx-preserving radiochemotherapy [7,8].

Based on the high efficacy of PD-1 inhibitors in the recurrent/metastatic situation, the CheckRad-CD8 trial studied the addition of immunotherapy to induction chemotherapy to select patients with locally advanced HNSCC for chemotherapy-free definitive radioimmunotherapy. In the CheckRad-CD8 trial patients with increasing intratumoral CD8+ immune cells in the surgical re-biopsy compared to baseline and patients with pathologic complete response (pCR) after induction chemo-immunotherapy were selected for radioimmunotherapy [9,10].

Several approaches were made to establish predictive biomarkers in HNSCC. Immunohistochemistry of PD-L1 was established in clinical trials, whereas for chemoimmunotherapy (platinum/5-flurouracil/pembrolizumab) the objective response rates in tumors with PD-L1 CPS <1, PD-L1 CPS ≥ 1 and PD-L1 CPS ≥ 20 tumors were 35.6 %, 36.4 % and 42.9 %, respectively, which is far from an optimum patient selection [1,11,12]. Further biomarker approaches such as tumor mutational burden (TMB), genetic signatures or immunologic active danger signals have potential predictive value but are not suitable for clinical use [13–16].

The pre-planned translational research program of the CheckRad-CD8 trial contained the analysis of the peripheral blood immune phenotype for the prediction and identification of good responders to induction chemo-immunotherapy. In this study, we analyzed the large dataset obtained by this multiparameter flow cytometric approach using a combination of different machine learning and ensemble algorithms. We further provide a strategy to determine the predictive power of pre-, post- and pre/post-comparison data to determine the probability of pCR and therefore, therapy monitoring concepts.

Material and methods

Clinical trial design and pre-planned translational reseach

The CheckRad-CD8 trial is a single-arm multicenter phase II study. Patients with histologically confirmed HNSCC stage III-IVB (according to TNM 8th edition) of the oral cavity, oropharynx, hypopharynx or supraglottic larynx were enrolled in this trial [17]. Treatment consisted of a single cycle of induction chemo-immunotherapy with cisplatin 30mg/m^2 body surface area (BSA) on days 1-3 and docetaxel 75mg/m^2 BSA on day 1. Tremelimumab (anti-CTLA4) fixed dose of 75mg and durvalumab (anti-PDL1) fixed dose of 1500 mg were both administered on day 5. Restaging assessment consisted of diagnostic imaging and endoscopy including representative re-biopsy of the primary tumor area was performed on day 22-26. Patients with an increase of intratumoral CD8+ cells of at least 20 % compared to baseline or without residual tumor in the re-biopsy defined as pathologic complete response (pCR) continued study treatment.

Further study treatment consisted of radiotherapy up to a cumulative dose of 70.0/63.0/54.0 Gy (tumor, involved neck, elective neck) delivered in 35 fractions by intensity-modulated radiation therapy. Immunotherapy continued with additional three cycles of durvalumab/tremelimumab followed by eight cycles of durvalumab monotherapy administered concomitant and subsequent to radiotherapy every fourth week.

The preplanned translational research program contained peripheral blood immune phenotyping (IPT) at several defined time points during study treatment according to previously optimized protocols [18,19]. This analysis investigates the value of peripheral blood immune cells to predict pCR after induction chemoimmunotherapy by using different machine learning and ensemble algorithms. The immune status of the patients was analyzed in this study pre-treatment (baseline, T1) and at the time point of re-staging after induction chemoimmunotherapy (T2) before entering radioimmunotherapy. In order to assess not only absolute cell numbers at the distinct time points, but also changes of immune cells, four different approaches were chosen: cell counts pre-induction (T1), cell counts post-induction (T2), the difference of cell counts pre- and post-induction (T2-T1), the absolute difference of cell counts pre- and post-induction (|T2-T1|). The latter was included to account for relevant changes when the direction of change is not important. The chosen study approach is visualized in Fig. 1.

Trial oversight

The clinical trial was registered with ClinicalTrials.gov (ClinicalTrials.gov ID NCT03426657). The leading institutional review board at the Friedrich-Alexander-Universität Erlangen-Nürnberg (number: 131_18 Az) and all institutional review boards approved the trial including the translational research program. All patients gave written informed consent before enrollment. The trial is an investigator-sponsored trial (IST).

Peripheral blood immune phenotyping technique

The detailed immune phenotyping (IPT) was performed by multicolor flow cytometry, which allows the analysis of numerous immune cell types in whole blood at once. The immune cells are identified in terms of their type, number and their activation state by the expression of certain cell surface proteins, as previously performed by our group for a prospective development and validation of a liquid immune profile-based signature (LIPS) to predict response of patients with recurrent/metastatic cancer to immune checkpoint inhibitors [16].

Endpoint of the biomarker analysis

The endpoint of this biomarker analysis was pCR in the re-biopsy after induction chemoimmunotherapy. Biopsies were obtained by an experienced otolaryngologic oncologist based on either the visible

residual tumor or the originally documented and imaged tumor area. Pathologic response was assessed in central pathology. All specimens were stained with hematoxylin-eosin and assessed by two experienced pathologists (i.e. M.E. and A.H.). To ensure sufficient coverage of a former tumor bed, relevant resorptive inflammation together with granulation and scar tissue was required in the post-induction biopsies. In order to make sure that residual tumor is not overseen, six section levels per specimen were evaluated.

Machine learning-based data analysis methods

Machine learning methods were used to predict the pCR probability based on the IPT data at different time points or combinations (see above and Fig. 1). All data were inspected manually to avoid any artifacts. The data contains 69, 63 and 61 patients for T1, T2, and T2-T1 or |T2-T1|, respectively. We performed stratified cross-validation with eight patients in the test set. We used univariate feature selection followed by principal component analysis to rebase the remaining features, i.e. the cell counts obtained from the IPT, on a linear combination of orthogonal principal components. These pre-processed data were used to fit classifiers, namely linear discriminant analysis (LDA), support vector machines (SVM), logistic regression (LR), random forests (RF), decision trees (DT) and gradient boosting techniques (XGBoost). For each classifier, we performed an intense hyperparameter search including the number of selected features, the number of principal components, and classifier-specific tuning parameters. Classifier-specific hyperparameters are shown in Supplementary Table 3. As evaluation criteria, we used confusion matrix metrics, namely true positive rate, true negative rate, false positive rate, and false negative rate, and computed mean accuracy, standard deviation, and area under the curve (AUC) for receiver-operating curves (ROC). We finally used soft and hard voting ensemble strategies to improve the overall pCR prediction accuracy.

Results

Clinical results

Between September 2018 and May 2020, eighty patients were enrolled in the phase II CheckRad-CD8 trial in eight German centers. Data cut-off was January 17th, 2021. One patient did not receive any study treatment and was excluded from all analyses. Baseline characteristics of the patients have previously been reported [9]. The safety profile of induction chemoimmunotherapy only [10] and the entire treatment consisting of induction chemoimmunotherapy, radiotherapy and maintenance immunotherapy has also already been published [9]. After induction chemoimmunotherapy, 41 patients (52 %) developed pCR and 35 had residual tumor (44 %). In three patients (4 %) no re-biopsy was performed due to toxicity ($n = 2$) or COVID-19 disease ($n = 1$). Detailed information on clinical efficacy have been reported before [9].

Availability of blood samples and biopsy specimens

Post-induction biopsy specimens were available in 76 patients, which represents 100 % of the patients with re-biopsy. A blood sample for IPT was provided by 69 patients at T1 (pre-induction) and by 63 at T2, i.e. post-induction. Thus, the cohorts contained the following number of datasets: pre-induction (T1) $n = 69$, post-induction (T2) $n = 63$, difference post- and pre-induction (T2-T1) $n = 61$, absolute difference pre- and post-induction (|T2-T1|) $n = 61$.

Performance of different machine learning approaches

We first evaluated which classifiers are capable of predicting pCR given the pre-processed data. We found that in principle all classifier methods allow a prediction better than chance (Supplementary Table 1). In general, more complex algorithms allow a higher pCR prediction accuracy.

As shown in Fig. 2, by systematically varying the incorporated number of features using features proposed by univariate feature selection in decreasing importance and the number of used principal components (Fig. 2A), we found that the best accuracy was achieved with a low number of features and principal components. This suggests that further information adds more noise to the classifier than improving the prediction. The best accuracy was achieved by the immune parameters obtained from |T2-T1| (Fig. 2B). In general, we found that less than 30 parameters and at most 10 principal components are needed for highly accurate predictions.

Predictive peripheral blood immune biomarkers

The prediction of pCR before chemoimmunotherapy induction (i.e. T1) is of particular clinical interest for the selection of the most beneficial therapy approach for the individual patient. Using the strategy shown in Fig. 2, we found that IPT at T1 is indeed predictive at most of 77.8 % accuracy using a support vector machine as a classification model, closely followed by boosting algorithms (75.5 % accuracy). The IPT after immunotherapy (i.e. T2) is also predictive to a similar extent (up to 78.5 % accuracy) as for T1. A similar picture emerges when looking at the difference T2-T1. There, the maximum accuracy is 78.3 %. However, when computing the accuracy on the absolute difference |T2-T1|, the classifiers showed a top performance up to 84.0 % (logistic regression), followed by 82.8 % when using support vector machines. This suggests that undirected differences in the IPT panel between T2 and T1 are highly predictive for pCR events.

We further investigated if a combination of several classifiers increased the prediction accuracy (ensemble solutions). When using the six best predictive classifiers in a hard-voting ensemble, we gain a top mean accuracy of 87.0 %, when the 61 markers of the IPT were considered (Fig. 3).

In contrast, the voting accuracy of IPT data before induction therapy resulted only in a maximum accuracy of 79.0 % with 43 biomarkers

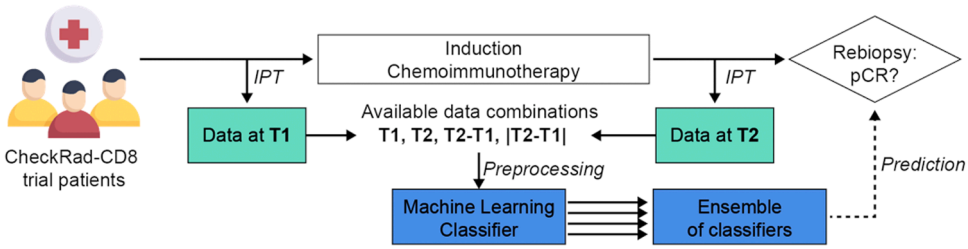

Fig. 1. Study approach. CheckRad-CD8 trial patients underwent immune phenotyping of the peripheral blood before and after chemoimmunotherapy induction yielding data at time point T1 and T2, respectively. Either the data at T1 or T2 alone, or their combination using their (absolute) difference in cell count values were used after preprocessing in machine learning-based classifiers to predict the pathological complete responder event (pCR). We additionally evaluated ensemble solutions to achieve a higher predictive power by combining several "weaker" classifiers.

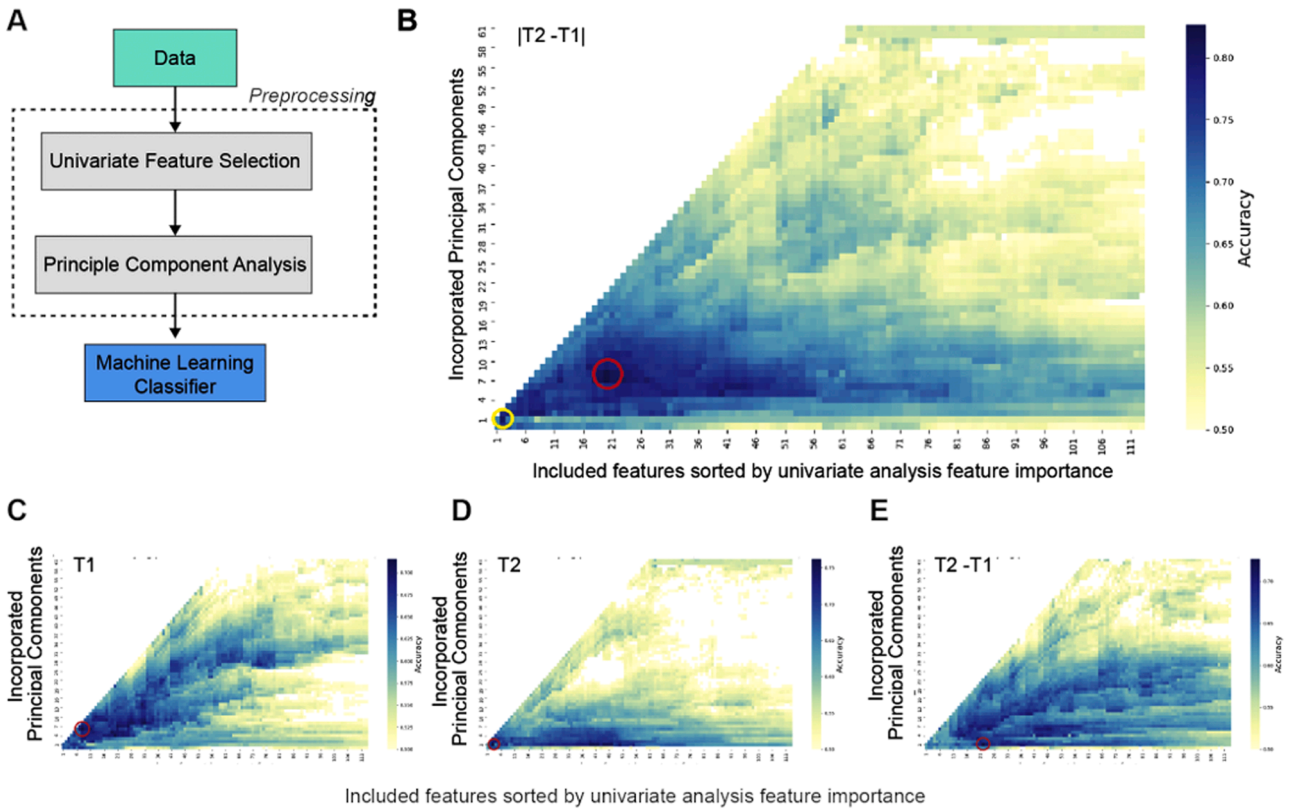

Fig. 2. Low dimensional feature representations allow accurate pCR prediction. A) Preprocessing pipeline for each dataset generated in Fig. 1. After univariate feature selection, principal component analysis was performed. The newly transformed vectors are used for Machine Learning. B) Classification accuracy heatmap of used features from univariate feature selection against used principal components for |T2-T1|. Red circle indicates maximum accuracy, yellow circle high accuracy with only a single principal component. C) – E) same as panel B) but for T1, T2 and T2-T1, respectively.

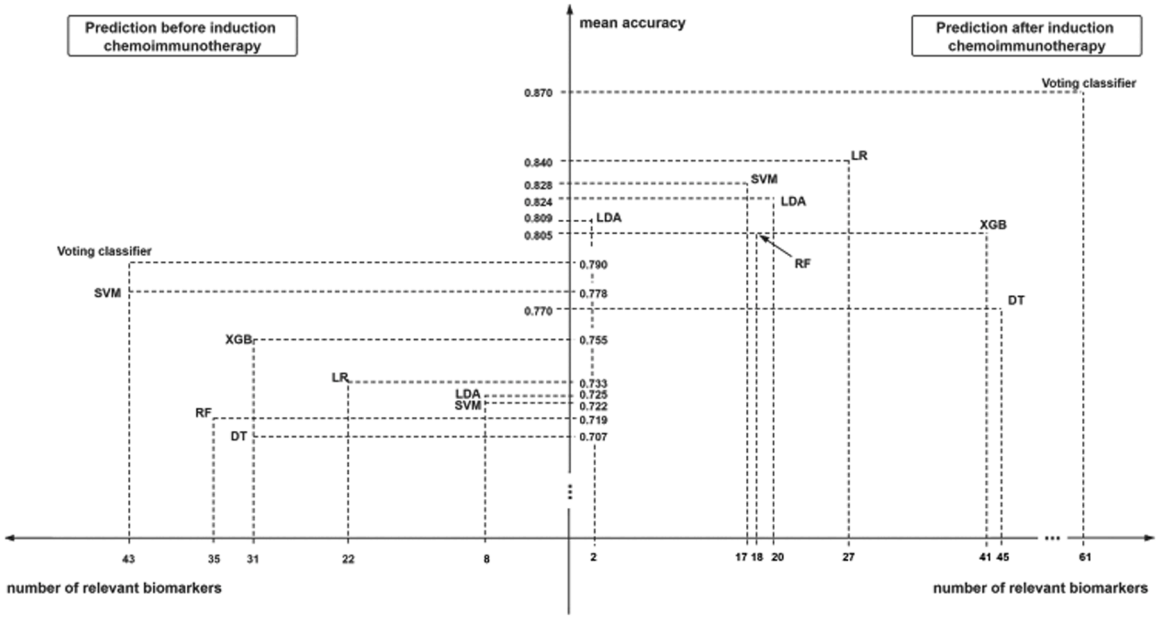

Fig. 3. Classifier comparison. The graph shows the relation of incorporated biomarkers (x-axis) and the yielded mean accuracy (y-axis) for a given classifier in relation to before the chemoimmunotherapy induction (left) or after (right). It is worth noting that the overall prediction accuracy is higher after the induction therapy than before.

being considered. Interestingly, the ensemble strategy with the highest accuracy incorporates mainly four classifiers trained on the absolute difference dataset, and two classifiers on the relative difference (XGBoost and decision trees), with 61 biomarkers being included.

Next, we asked which of the features are (I) predictive for pCR and (II) are overlapping with the dataset groups. As visualized in Fig. 4, across several datasets, cells of the innate immune system are most prominent here.

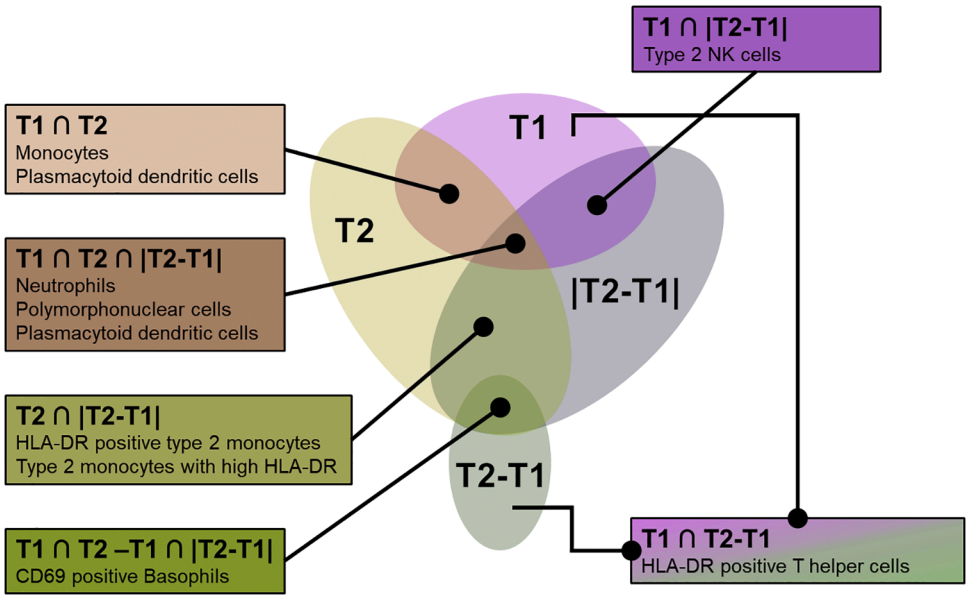

Fig. 4. Identified peripheral blood immune biomarkers with predictive value. Overlap of biomarkers with high predictive power across the investigated datasets. Notably, especially cells of the innate immune system cells are highly represented.

For T1, T2 and |T2-T1| polymorphonuclear cells (PMN) *per se*, neutrophils, and plasmacytoid dendritic cells (pDCs) had high predictive power. Regarding T1 and T2, again pDCs and additionally monocytes have to be considered. The expression of HLA-DR on the latter innate immune cells is predictive when being analyzed at T2 or calculated by |T2-T1|, while natural killer (NK) cells are prominent for T1 and |T2-T1| with this regard. Again PMN, but now basophils have predictive value for pCR for T1, T2-T1 and |T2-T1|. Only one subpopulation of adaptive immune cells (HLA-DR positive T helper cells) was predictive at T1 and T2-T1 (Fig. 4). An overview of the biomarker feature importance for top performing classifiers for the relevant T1 and |T2-T1| cases is shown in Supplementary Table 2.

Discussion

Prediction of therapy response to immunotherapies is still a big challenge, not only for HNSCC [20]. In this study, we have assessed the potential of parameters obtained from detailed IPT of the peripheral blood of patients with HNSCC who were treated within the CheckRad-CD8 trial to predict pathological pCR events using standard methods of Machine Learning. We found that especially cells of the innate immune system are features being essential for highly predictive Machine Learning models.

Low-dimensional space is beneficial for pCR prediction

The detailed analyses revealed that a low dimensional representation of the data leads to better results than using the raw measurements available (Fig. 2 and Table 1). This is consistent with previous reports on high dimensionality [21–23]. The sophisticated data preprocessing allows a multitude of machine learning methods to achieve high classification accuracy (Supplementary Table 1). However, we also detected a trend that a higher machine learning method complexity coincides with higher prediction accuracy (Table 1, Fig. 3), whereas we also find that a low amount of features are already sufficient to predict pCR above chance levels (Figs. 2 and 3).

Early prediction of pCR using routine blood tests

Early prediction of pCR for a specific patient cohort that allows a tailored therapy monitoring strategy is needed to increase patient care and reduce economic burden in the clinic [24]. In previous investigations, we identified histological parameters such as intratumoral CD8+ immune cells and PD-L1 expression on immune cells to be associated with pCR in HNSCC patients [10]. However, considering the dynamics of immune changes longitudinal monitoring of changes of immune cells and markers should be envisaged by analyzing easily available blood-based immune markers [16]. We identified that the peripheral blood immune status of the patients at T1, i.e. before induction chemoimmunotherapy, is capable of predicting pCR at an accuracy of almost 80 %. It can be expected that future studies will further increase the detection accuracy.

Predictive potential of innate immune cells in induction chemoimmunotherapy of HNSCC Patients

This prospective translational research approach identified innate immune cells as most relevant predictors of treatment response to combined induction chemo-immunotherapy with immune checkpoint inhibitors in HNSCC. Particularly polymorphonuclear cells and their subpopulations such as neutrophils and basophils, NK cells, Monocytes, and pDCs are present across several datasets. It has become obvious that high neutrophil counts are a negative predictor of tumour responses to immune checkpoint inhibitor-based immune therapies [24]. In this regard, this study is the first to add new findings that the magnitude of the changes of the investigated immune cells pre- to post- induction chemoimmunotherapy and their overall count before therapy are similarly effective for predicting pCR after induction chemoimmunotherapy in locally advanced HNSCC. Besides neutrophils, basophils, NK cells and monocytes were additionally identified as valuable markers of response predictions through machine learning methods. Based on retrospective analyses, Hiltbrunner et al. showed that increased basophil counts were associated with increased tumour size reduction, concomitantly with the development of an irAE in metastatic non-small cell lung cancer patients treated with immune checkpoint inhibitors [25]. Translational studies further revealed that even before the start of radiochemotherapy, basophil levels of patients with brain tumours suffering from HCMV-associated encephalopathy are significantly lower compared with those who were not [26]. Pre-treatment neutrophil to lymphocyte ratio has been shown to be prognostic among stage III NSCLC patients treated with adjuvant immunotherapy, and may serve as a predictive biomarker of immunotherapy benefit [27]. In this regard, we showed here for the first time that both, neutrophils and basophils have to be

Table 1
Description of Peripheral blood immune cell biomarkers which were used in different approaches.

Data set 1
PMN (polymorphonuclear cells)
Neutrophils
CD123 low+ basophils
CD25+ eosinophils
CD25+ basophils
HLA-DR+ eosinophils
Monocytes
Classical monocytes (type 1)
Non-classical monocytes (type 3)
DCs
Plasmacytoid DCs (P02)
Plasmacytoid DCs (P04)
Myeloid DCs type 1
Myeloid DCs type 2
NK cells type 2
NK cells type 3
CD25+ NK cells
HLA-DR high+ NK cells
HLA-DR+ B cells
CD69+ B cells
Tregs
CD8 low T Killer cells
Double positive T cells
CD25+ T Helper cells
HLA-DR+ T Helper cells
HLA-DR high+ T Helper cells
CD69+ T cells
CD69+ T Killer cells
CD69+ T Helper cells
CTLA4+ T cells
CTLA4+ T Helper cells
CTLA4+ T Killer cells
CTLA4+ naïve T Helper cells
PD1+ T Helper cells
PD1⁺ effector memory T Helper cells
PD1+ naïve T Killer cells
PD1+ effector T Killer cells
T Killer / Treg ratio

Data set 2
PMN (polymorphonuclear cells)
Neutrophils
CD69+ basophils
Monocytes
Classical monocytes (type 1)
intermediate monocytes (type 2)
HLA-DR+ intermediate monocytes (type 2)
HLA-DR high+ intermediate monocytes (type 2)
Plasmacytoid DCs (P02)
Plasmacytoid DCs (P04)
PD1+ T Killer cells

Data set difference
CD69+ basophils
NK cells type 1
HLA-DR+ T Helper cells

Data set absolute difference
PMN (polymorphonuclear cells)
Neutrophils
CD69+ basophils
HLA-DR+ intermediate monocytes (type 2)
HLA-DR+ non-classical monocytes (type 3)
HLA-DR high+ intermediate monocytes (type 2)
HLA-DR high+ non-classical monocytes (type 3)
CD69+ monocytes
Plasmacytoid DCs (P02)
NK cells type 2
T cells
T Helper cells (P02)
T Killer cells (P03)
Double negative T cells (P02)
CD25 high+ T Helper cells

considered for prediction of responses to induction chemo-immunotherapy in locally advanced HNSCC. In addition, pDCs and NK cells should also be taken into account. As already shown within the ST-ICI trial (ClinicalTrials.gov ID NCT03453892) where 54 peripheral blood immune markers were included in a univariate cox survival analysis, pDCs and NK cells are part of the 14 immune cell subtypes which are associated with overall survival of HNSCC and NSCLC patients who were treated with immune checkpoint inhibitors [16]. Thus, these immune biomarkers have proven their suitability to predict pCR following induction chemoimmunotherapy in HNSCC. Combined with standard machine learning methods they are potential candidates for future standard analyses of blood samples, rendering our approach a low-cost application for therapy monitoring decisions. We conclude that the undirected alteration of the innate immune cell distribution in the peripheral blood due to induction chemoimmunotherapy is predictive for pCR in HNSCC. This interesting fact, i.e. that the alteration is undirected, can be the result of the presence of multiple modulating events at once, such as systemic responses, bone marrow turnover and mobilization, and homeostatic disruption. It has been reported previously that white blood cell counts can vary largely dynamically after full body radation [28]. Further, mathematical simulation studies of chemo-immunotherapy have shown that multiple cell count responses after induction are possible, being inconclusive in the direction of each white blood cell type [29]. Even if global trends are conclusive, such as a decline of immune cells after radiation [28], we are only observing two short snapshots of the process. Our findings together with these studies

suggest, that there the response is highly dynamic and dependent of multiple factors, where the change of the system is more predictive than the system itself.

Data availability

Trial data are posted on clinicaltrials.gov as required. Access to supporting documents such as the protocol and informed consent will be made available on reasonable request. The participant data are uploaded at zenodo platform (https://zenodo.org/records/10145138).

Authors contributions

M.H. project administration, conceptualization, acquisition, investigation, writing, reviewing and editing **B.F.** project administration, conceptualization, acquisition, investigation, visualization, writing, reviewing and editing **U.S.G.** project administration, supervision, writing, reviewing and editing **X.T.** investigation, formal analysis, data curation **M.E.** formal analysis, investigation, methodology, writing, reviewing **A.J.D.** formal analysis, acquisition, investigation, writing, reviewing and editing **G.K.** acquisition, data curation, reviewing **T.I.** acquisition, data curation, reviewing **M.F.** acquisition, data curation, reviewing **S.L.** acquisition, data curation, reviewing **M.G.H.** acquisition, data curation, reviewing **B.T.** acquisition, data curation, reviewing **T.B.B.** acquisition, data curation, reviewing **I.B.** formal analysis, acquisition, reviewing **J.G.Z.** formal analysis, validation, writing **A.Ha.** formal analysis, investigation, methodology, supervision **R.F.** conceptualization, funding, supervision, writing, reviewing and editing **H.I.** acquisition, data curation, supervision, reviewing **M.D.** investigation, data curation, formal analysis, supervision, writing, reviewing and editing **A.O.G.** acquisition, data curation, formal analysis, writing, reviewing and editing **A.M.K**. investigation, data curation, methodology, formal analysis, visualization, writing, reviewing and editing

Declaration of competing interest

M.H. conflict of interest with Merck Serono (advisory role, speakers' bureau, honoraria, travel expenses, research funding); MSD (advisory role, speakers' bureau, honoraria, travel expenses, research funding); AstraZeneca (advisory role, speakers' bureau, honoraria, travel expenses, research funding); Novartis (research funding); BMS (advisory role, speakers' bureau, honoraria, travel expenses, research funding); Teva (travel expenses); Sanofi (advisory role, honoraria).

M.E. conflict of interest with Diaceutics (employment, honoraria, advisory role, speakers' bureau, travel expenses); Cepheid (research funding, advisory role); AstraZeneca (honoraria, advisory role, speakers' bureau, travel expenses); Roche (honoraria, travel expenses); MSD (honoraria, speakers' bureau); GenomicHealth (honoraria, advisory role, speakers bureau, travel expenses); Astellas (honoraria, speakers' bureau); Janssen-Cilag (honoraria, advisory role, research funding, travel expenses); Stratifyer (research funding, patents).

G.K. conflict of interest with BMS (advisory role); Lilly (advisory role); Roche (advisory role)

S.L. conflict of interest with AstraZeneca (honoraria, advisory role); BMS (honoraria, advisory role, speakers' bureau); MSD (honoraria, advisory role); Merck Serono (honoraria, speakers' bureau); ISA-Pharmaceuticals (research funding)

M.G.H. conflict of interest with Roche (stock); Varian (stock); Sanofi (stock); AstraZeneca (honoraria); BMS (honoraria, advisory role); MSD (honoraria, advisory role); Merck Serono (honoraria); Celgene (honoraria).

B.T. conflict of interest with BMS (advisory role, honoraria); Merck Serono (advisory role, speakers' bureau, honoraria); MSD (advisory role, speakers' bureau, honoraria); Sanofi (advisory role, honoraria).

A.Hi. conflict of interest with Roche (honoraria).

A.H. conflict of interest with BMS (honoraria, advisory role); MSD (honoraria, advisory role); Roche (honoraria, advisory role, research funding); AstraZeneca (honoraria, advisory role, research funding); Boehringer Ingelheim (honoraria); Abbvie (honoraria); Cepheid (advisory role, research funding); Quiagen (advisory role); Janssen-Cilag (honoraria, advisory role, research funding); Ipsen (honoraria, advisory role); NanoString Technologies (advisory role, research funding, expert testimony); Illumina (advisory role); 3DHistech (advisory role); Diaceutics (advisory role); BioNTech (research funding). W.B. conflict of interest with BMS (advisory role); MSD (advisory role); Merck Serono (advisory role); Pfitzer (advisory role); AstraZeneca (advisory role).

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All other not listed authors do not have a conflict of Interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neo.2023.100953.

References

[1] B. Burtness, K.J. Harrington, R. Greil, D. Soulieres, M. Tahara, G. de Castro Jr., A. Psyrri, N. Baste, P. Neupane, A. Bratland, et al., Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study, Lancet 394 (2019) 1915–1928, https://doi.org/10.1016/S0140-6736(19)32591-7.

[2] R.L. Ferris, G. Blumenschein Jr., J. Fayette, J. Guigay, A.D. Colevas, L. Licitra, K. Harrington, S. Kasper, E.E. Vokes, C. Even, et al., Nivolumab for recurrent squamous-cell carcinoma of the head and neck, N. Engl. J. Med. 375 (2016) 1856–1867, https://doi.org/10.1056/NEJMoa1602252.

[3] R. Fietkau, M. Hecht, B. Hofner, D. Lubgan, H. Iro, O. Gefeller, C. Rodel, M. G. Hautmann, O. Kolbl, A. Salay, et al., Randomized phase-III-trial of concurrent chemoradiation for locally advanced head and neck cancer comparing dose reduced radiotherapy with paclitaxel/cisplatin to standard radiotherapy with fluorouracil/cisplatin: The PacCis-trial, Radiother. Oncol. 144 (2020) 209–217, https://doi.org/10.1016/j.radonc.2020.01.016.

[4] P. Blanchard, J. Bourhis, B. Lacas, M.R. Posner, J.B. Vermorken, J.J. Cruz Hernandez, A. Bourredjem, G. Calais, A. Paccagnella, R. Hitt, et al., Taxane-cisplatin-fluorouracil as induction chemotherapy in locally advanced head and neck cancers: an individual patient data meta-analysis of the meta-analysis of chemotherapy in head and neck cancer group, J. Clin. Oncol. 31 (2013) 2854–2860, https://doi.org/10.1200/JCO.2012.47.7802.

[5] J.L. Lefebvre, G. Andry, D. Chevalier, B. Luboinski, L. Collette, L. Traissac, D. de Raucourt, J.A. Langendijk, Head E, Neck Cancer G, Laryngeal preservation with induction chemotherapy for hypopharyngeal squamous cell carcinoma: 10-year results of EORTC trial 24891, Ann. Oncol.: Off. J. Eur. Soc. Med. Oncol. /ESMO 23 (2012) 2708–2714, https://doi.org/10.1093/annonc/mds065.

[6] W. Budach, E. Bolke, K. Kammers, P.A. Gerber, K. Orth, S. Gripp, C. Matuschek, Induction chemotherapy followed by concurrent radio-chemotherapy versus concurrent radio-chemotherapy alone as treatment of locally advanced squamous cell carcinoma of the head and neck (HNSCC): A meta-analysis of randomized trials, Radiother. Oncol. 118 (2016) 238–243, https://doi.org/10.1016/j.radonc.2015.10.014.

[7] S. Semrau, M. Haderlein, D. Schmidt, M. Lell, W. Wolf, F. Waldfahrer, M. Uder, H. Iro, T. Kuwert, R. Fietkau, Single-cycle induction chemotherapy followed by chemoradiotherapy or surgery in patients with head and neck cancer: what are the best predictors of remission and prognosis? Cancer 121 (2015) 1214–1222, https://doi.org/10.1002/cncr.29188.

[8] S. Semrau, D. Schmidt, M. Hecht, M. Haderlein, C. Kitzsteiner, S. Muller, M. Traxdorf, A. Agaimy, H. Iro, T. Kuwert, 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. 100 (2020) 104479, https://doi.org/10.1016/j.oraloncology.2019.104479.

[9] M. Hecht, M. Eckstein, S. Rutzner, J. von der Grun, T. Illmer, G. Klautke, S. Laban, M.G. Hautmann, T.B. Brunner, B Tamaskovics, 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 10 (2022), https://doi.org/10.1136/jitc-2021-003747.
[10] M. Hecht, A.O. Gostian, M. Eckstein, S. Rutzner, J. von der Grun, T. Illmer, M. G. Hautmann, G. Klautke, S. Laban, T. Brunner, 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 8 (2020), https://doi.org/10.1136/jitc-2020-001378.
[11] B. Burtness, K. Harrington, R. Greil, D. Soulières, M. Tahara, G. De Castro, A. Psyrri, N.B. Rotllan, P. Neupane, Å. Bratland, KEYNOTE-048: Phase III study of first-line pembrolizumab (P) for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC), Ann. Oncol. 29 (2018) viii729.
[12] D. Rischin, K.J. Harrington, R. Greil, D. Soulieres, M. Tahara, G. de Castro, A. Psyrri, N. Baste, P.C. Neupane, A. Bratland, Protocol-specified final analysis of the phase 3 KEYNOTE-048 trial of pembrolizumab (pembro) as first-line therapy for recurrent/metastatic head and neck squamous cell carcinoma (R/M HNSCC), American Society of Clinical Oncology, 2019. Editor (ed)˙(eds)City.
[13] K Clasen, S Welz, H Faltin, D Zips, F Eckert, Dynamics of HMBG1 (High Mobility Group Box 1) during radiochemotherapy correlate with outcome of HNSCC patients, Strahlenther. Onkol. 198 (2022) 194–200, https://doi.org/10.1007/s00066-021-01860-8.
[14] M. Jakob, K. Sharaf, M. Schirmer, M. Leu, S. Kuffer, M. Bertlich, F. Ihler, F. Haubner, M. Canis, J. Kitz, Role of cancer stem cell markers ALDH1, BCL11B, BMI-1, and CD44 in the prognosis of advanced HNSCC, Strahlenther. Onkol. 197 (2021) 231–245, https://doi.org/10.1007/s00066-020-01653-5.
[15] N.I. Vokes, D. Liu, B. Ricciuti, E. Jimenez-Aguilar, H. Rizvi, F. Dietlein, M.X. He, C. A. Margolis, H.A. Elmarakeby, J Girshman, et al., Harmonization of Tumor mutational burden quantification and association with response to immune checkpoint blockade in non-small-cell lung cancer, JCO Precis Oncol. 3 (2019), https://doi.org/10.1200/PO.19.00171.
[16] JG Zhou, A.J. Donaubauer, B. Frey, I. Becker, S. Rutzner, M. Eckstein, R. Sun, H. Ma, P. Schubert, C. Schweizer, et al., Prospective development and validation of a liquid immune profile-based signature (LIPS) to predict response of patients with recurrent/metastatic cancer to immune checkpoint inhibitors, J. Immunother. Cancer 9 (2021), https://doi.org/10.1136/jitc-2020-001845.
[17] M. Hecht, A.O. Gostian, M. Eckstein, S. Rutzner, J. von der Grün, T. Illmer, M. G. Hautmann, T. Brunner, S. Laban, G. Klautke, et al., Single cycle induction treatment with cisplatin/docetaxel plus durvalumab/tremelimumab in stage III-IVB head and neck squamous cell cancer (CheckRad-CD8 trial), Ann. Oncol. 30 (2019).
[18] A.J. Donaubauer, I. Becker, P.F. Ruhle, R. Fietkau, U.S. Gaipl, B. Frey, Analysis of the immune status from peripheral whole blood with a single-tube multicolor flow cytometry assay, Methods Enzymol. 632 (2020) 389–415, https://doi.org/10.1016/bs.mie.2019.03.003.
[19] A.J. Donaubauer, P.F. Ruhle, I. Becker, R. Fietkau, U.S. Gaipl, B. Frey, One-Tube Multicolor Flow Cytometry Assay (OTMA) for Comprehensive Immunophenotyping of Peripheral Blood, Methods Mol. Biol. 1904 (2019) 189–212, https://doi.org/10.1007/978-1-4939-8958-4_8.
[20] J.C. Park, H.N. Krishnakumar, S.V. Saladi, Current and Future Biomarkers for Immune Checkpoint Inhibitors in Head and Neck Squamous Cell Carcinoma, Curr. Oncol. 29 (2022) 4185–4198, https://doi.org/10.3390/curroncol29060334.
[21] A.N. Bhagoji, D. Cullina, C. Sitawarin, P. Mittal, Enhancing robustness of machine learning systems via data transformations, IEEE, 2018, pp. 1–5. Editor (ed)˙(eds)City.
[22] G.T. Reddy, M.P.K. Reddy, K. Lakshmanna, R. Kaluri, D.S. Rajput, G. Srivastava, T. Baker, Analysis of dimensionality reduction techniques on big data, IEEE Access 8 (2020) 54776–54788.
[23] M. Verleysen, D. François, The curse of dimensionality in data mining and time series prediction, Springer, 2005, pp. 758–770. Editor (ed)˙(eds)City.
[24] C. Valero, M. Lee, D. Hoen, K. Weiss, D.W. Kelly, P.S. Adusumilli, P.K. Paik, G. Plitas, M. Ladanyi, M.A. Postow, et al., Pretreatment neutrophil-to-lymphocyte ratio and mutational burden as biomarkers of tumor response to immune checkpoint inhibitors, Nat. Commun. 12 (2021) 729, https://doi.org/10.1038/s41467-021-20935-9.
[25] S. Hiltbrunner, M.L. Spohn, R. Wechsler, D. Akhoundova, L. Bankel, S. Kasser, S. Bihr, C. Britschgi, M.H. Maathuis, A Curioni-Fontecedro, Comprehensive statistical exploration of prognostic (Bio-)markers for responses to immune checkpoint inhibitor in patients with non-small cell lung cancer, Cancers (Basel) 14 (2021), https://doi.org/10.3390/cancers14010075.
[26] N.L. Goerig, B Frey, K. Korn, B. Fleckenstein, K. Uberla, M.A. Schmidt, A. Dorfler, T. Engelhorn, I. Eyupoglu, P.F. Ruhle, et al., Early mortality of brain cancer patients and its connection to cytomegalovirus reactivation during radiochemotherapy, Clin. Cancer Res. 26 (2020) 3259–3270, https://doi.org/10.1158/1078-0432.CCR-19-3195.
[27] A.K. Bryant, K. Sankar, G.W. Strohbehn, L. Zhao, D. Elliott, A. Qin, S. Yentz, N. Ramnath, M.D. Green, Prognostic and predictive value of neutrophil-to-lymphocyte ratio with adjuvant immunotherapy in stage III non-small-cell lung cancer, Lung Cancer 163 (2022) 35–41, https://doi.org/10.1016/j.lungcan.2021.11.021.
[28] T.M. Fliedner, D. Graessle, V. Meineke, H. Dorr, Pathophysiological principles underlying the blood cell concentration responses used to assess the severity of effect after accidental whole-body radiation exposure: an essential basis for an evidence-based clinical triage, Exp. Hematol. 35 (2007) 8–16, https://doi.org/10.1016/j.exphem.2007.01.006.
[29] L. de Pillis, K. Renee Fister, W. Gu, C. Collins, M. Daub, D. Gross, J. Moore, B. Preskill, Mathematical model creation for cancer chemo-immunotherapy, Comput. Math. Methods Med. 10 (2009) 165–184, https://doi.org/10.1080/17486700802216301.