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REVIEW

Personalized biomarker-based treatment strategy for patients with squamous cell carcinoma of the head and neck: EORTC position and approach

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The molecular landscape of squamous cell carcinoma of the head and the neck (SCCHN) has been characterized and actionable or targetable genomic alterations have been identified. However, targeted therapies have very limited activity in unselected SCCHN, and the current treatment strategy is still based on tumor location and disease stage and not on tumor biology**.** Trying to select upfront the patients who will benefit from a specific treatment might be a way to improve patients' outcome. With the objective of optimizing the activity of targeted therapies and immunotherapy, we have designed an umbrella biomarker-driven study dedicated to recurrent and/or metastatic SCCHN patients (EORTC-1559-HNCG, NCT03088059). In this article, we review not only the different trial designs for biomarker-driven studies with their respective advantages and opportunities but also the potential pitfalls that led to the design of the EORTC-1559-HNCG protocol. We also discuss the scientific and logistic challenges of biomarker-driven trials.

Key words: SCCHN, biomarker, personalized, umbrella, EORTC-1559-HNCG

Introduction

Squamous cell carcinoma of the head and the neck (SCCHN) is the seventh most common malignancy [1]. The main risk factors are smoking and alcohol consumption, which are responsible for the majority of SCCHN occurring in the oral cavity, pharynx, and larynx. Another risk factor for oropharyngeal cancer (OPC) is the human papillomavirus (HPV). Tobacco and/or alcohol-induced SCCHN and HPV-related OPC are two separate entities with different clinical and molecular features [2–4].

Less than 60% of the patients with locally advanced SCCHN remain disease-free at 3 years, despite a multimodal treatment combining surgery and/or (chemo)radiation [5]. Patients with recurrent/metastatic disease who are not amenable to radiotherapy or surgery have a median survival of 10–12 months. Platinum-based chemotherapy in combination with cetuximab improves overall survival (OS) in the first-line treatment of incurable disease [6]. Nivolumab increases OS of patients who progress after platinum therapy [7]. Pembrolizumab is also approved in the same indication by the Food and Drug Administration (FDA) [8]. No standard of care exists for patients who progress after platinum-therapy and antiprogrammed cell death protein 1 (PD-1) compounds.

The current treatment strategy of patients with SCCHN is still based on tumor location and disease stage and not on tumor

Table 1. Advantages and pitfalls of 'biomarker-driven' clinical trial designs

Master protocols	Advantages	Disadvantages
Basket trials Histology agnostic	Can include rare cancer types Can target low incidence actionable/targetable molecular alterations	Assumes that molecular biology can replace histology and that a specific genetic alteration has the same signification across different tumor types
Umbrella trials Histology specific	Targets molecular alterations in one cancer type and avoid heterogeneity due to multiple cancer histologies Enables to get more conclusive results for one tumor type	Feasibility limited for rare cancers
Screening programs	Have the potential to identify an actionable/targetable genetic alteration Can facilitate the access to early development clinical trials	If an actionable/targetable alteration is present, the specific drug is not always available with the risk that a low number of patients finally benefits from this program
Strategy trials	Have the potential to identify an actionable/targetable genetic alteration	Effect of the strategy can be diluted by less effective target-drug pairs

biology [4, 9, 10]. Targeted therapies have shown disappointing results [11–13]. Trying to select upfront the patients who will benefit from a specific treatment might improve the outcome. The European Organization for Research and Treatment of Cancer (EORTC) is conducting the EORTC-1559-HNCG trial, the first international biomarker-driven umbrella trial in recurrent SCCHN. In this article, we will review not only the different trial designs for biomarker-driven studies with their respective advantages and opportunities but also the potential pitfalls that led to the design of the EORTC 1559 protocol. We will also discuss the scientific and logistic challenges of this trial.

Lessons learned from previous biomarker-driven studies

Study designs

'Master protocol' terminology refers to a framework in which several (sub)studies that investigate multiple therapies are operated in parallel under one 'overarching' master protocol [14]. Master protocols include two different study designs: basket and umbrella trials. Table 1 summarizes the opportunities and drawbacks of these designs.

'Basket trials' are biomarker-driven clinical trials that include patients based on pre-defined specific molecular tumor abnormalities, irrespective of tumor origin and histology (Table 2). One of the advantages of this histology agnostic approach is to investigate the activity of targeted drugs across different cancer types, even in rare cancers for which clinical trials do not exist. They also offer the possibility to target low incidence molecular alterations.

'Umbrella trials' are biomarker-driven clinical trials that are histology-specific, investigating different therapeutic interventions in a single cancer type (Table 3). A histology-specific approach is interesting to avoid the heterogeneity due to different biology across various tumor types.

'Strategy trials' investigate if selecting the treatment based on molecular alterations results in superior outcome compared with standard therapy, independently of the drug, the disease, and the studied biomarker(s).

'Molecular screening programs' have been implemented to facilitate the access to precision medicine trials. These screening initiatives can be histology-agnostic or histology-specific.

Theranostic and molecular screening tools

Different diagnostic tests are routinely used to predict the activity or resistance of some targeted therapies. Most of them are evaluated on tumor biopsies, although liquid biopsies are entering into the clinic [e.g. epidermal growth factor receptor (*EGFR) T790M* mutation in non-small-cell lung cancers (NSCLC)]. Biomarkers can be evaluated not only at the proteomic level such as the estrogen receptor status assessed by immunohistochemistry (IHC) but also at the genomic level such as Human Epidermal Receptor-2 (*HER2)* amplifications or *EGFR* activating mutations.

The tumor molecular profile has been obtained in 74%–93% of screened patients in biomarker-driven clinical trials [16, 18–23]. Most of them use DNA sequencing on tumor biopsies. Reproducibility and reliability of the molecular screening tools are important. Most of the trials use certified laboratories, but the analysis is not always centralized. In these cases, some trials carried out an interlaboratory analytical validation before starting the trial [24] or validated the assay [25].

A fresh biopsy is probably more reliable than an archival one. Indeed, the cancer molecular profile can change during disease evolution [26]. IMPACT [18, 21] used archival formalin-fixed paraffin-embedded (FFPE) tissue. In the LUNG-MAP trial [27] and LUNG-MATRIX trial [23], both archival or fresh-taken tissues are accepted. In the MOSCATO 01 [20], NCI-MPACT [15], NCI-MATCH [15], BATTLE [16], and SHIVA [17] trials, a fresh tumor biopsy has/had to be taken for the trial purpose.

Actionable genomic alteration frequency and enrolment rate

According to the ESMO glossary [28], 'targetable genomic alteration' encodes an altered protein against which a drug exists or can be synthesized and an 'actionable genomic alteration'

Table 2. Selected histology agnostic biomarker-driven approaches

Study	Tumor	Study design	Biomarker	Methodology	End point	Identification of target and number of treated patients	Results and impact on outcome
IMPACT [18, 21]	All, refractory advanced cancer	Screening program	Archival (FFPE) PCR-based sequencing for selected genes (*PIK3CA, BRAF, KRAS and NRAS, EGFR, KIT, GNAQ, TP53 and MET*), Sanger sequencing for *RET* analysis, IHC for PTEN loss of expression and FISH for *ALK* translocation Update 2017: Sequencing by NGS at MD Anderson (11, 46 or 50 genes depending on the panel), Foundation Medicine (182 genes), Knight Diagnostics (48 genes) or other CLIA-certified laboratories ! Gene panels of different sizes were used for MP!	Screening route to phase I Assignment to phase I clinical trial based on the identification of MA	Clinical outcome of pts with MA treated with matched therapy versus pts not treated with matched therapy	• 1144/1283 pts had adequate tissue for molecular analysis (89.2%) • 460/1144 analyzed pts had 1 or more MA (40.2%) • 211/460 (45.8%)treated with matched therapy=16.4% of total population Update 2017: • 1179/1436 pts had 1 or more MA (82%) • 914/1179 had 1 or more targetable alteration (77.5%) • 390/637 (45.8%) pts with at least 1 alteration that were treated, received matched therapy=27% of total population	Analysis on 379 with 1 MA: 175 pts treated with matched therapy versus 116 nonmatched (88 pts excluded from clinical outcome analysis): • ORR: 27% in matched therapy versus 5% (P<0.0001) • SD ≥6 months: 23% versus 10% • OS: 13.4 versus 9 months (P=0.017) Update 2017: • ORR: 11% versus 5% (*P* =0.0099) • SD≥6 months+CR+PR: 29% versus 24% • FFS: 3.4 versus 2.9 months (*P*=0.0015) • - OS: 8.4 versus 7.3 months (*P*=0.41)
SHIVA trial [17]	All, refractory advanced cancer	Strategy trial Multicenter, open-label, proof-of-concept, randomized, phase II trial	New biopsy Mutations by targeted NGS (AmpliSeq cancer panel) CNA by Affymetix IHC for estrogen, progesterone and androgen receptors	pts with MA in one of the 3 molecular pathways that could be matched with 11 different targeted agents were randomized between the targeted therapy and control arm	PFS	• 716/741 screened pts underwent tumor sample • 293/741 screened patients had at least 1 MA matching one therapy (40%) • - 196/741 pts were randomized (26%)	Negative trial: Median PFS was 2.3 months in the experimental group versus 2.0 months in the control group (*P*=0.41)

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Table 2. *Continued*

Study	Tumor	Study design	Biomarker	Methodology	End point	Identification of target and number of treated patients	Results and impact on outcome
MOSCATO 01 trial [20]	All, advanced cancer	Screening program Single-center, singe-arm, open-label, prospective clinical trial	New biopsy (Fresh-frozen) At the start of trial: targeted sequencing (first Ion Ampliseq Cancer Panel covering 40 genes, then the Ion Ampliseq Cancer Hotspot Panel v2.0 in 50 genes and finally an Ion AmpliSeq custom design covering 75 genes) aCGH analysis and IHC for phospho-MET RNA sequencing and whole-exome sequencing were added during the trial	Screening route to phase I/II Assignment to phase I clinical trial based on the identification of MA	Evaluate the clinical benefit as measured by percentage of pts presenting PFS on matched therapy (PFS2) 1.3-fold longer than the PFS on prior therapy (PFS1)	• 948/1035 included pts underwent biopsy • MP obtained in 843/948 pts (89%) • 411/843 pts had a MA (49%) • - 199 pts were treated with a targeted therapy =19% of total population	PFS2/PFS1 ratio >1.3 in 63/199 pts treated with targeted therapy (33%)=7% of successfully screened pts
CREATE trial [60–63]	Advanced tumors characterized by *MET* and/or *ALK* alterations (papillary renal-cell carcinoma type 1, alveolar soft part sarcoma, clear-cell sarcoma, anaplastic large-cell lymphoma, inflammatory myofibroblastic tumour, and alveolar rhabdomyosarcoma)	Multinational, multitumor, prospective phase II clinical trial	Tumour containing tissue block (FFPE) from the primary tumour and/or metastatic site: sequencing (bidirectional Sanger sequencing method of only 1 gene) (*MET*), FISH for copy number status	Treatment with crizotinib in the different patient cohorts	ORR	No biomarker-positivity needed for entering the trial	Results published per histology
NCI-MPACT [15]	All, advanced solid tumor	Strategy trial Double-blind, randomized trial	New biopsy NGS of > 380 unique actionable variants in 20 genes	Pts with specific mutation are randomized in 2 : 1 ratio to receive targeted therapy versus control (not specifically targeting the detected mutation/pathway of interest)	ORR and 4-month PFS	NA, 270 assessable pts are planned for enrollment.	Over 100 patients have been screened to date, though no interim analysis results have been presented to date
NCI-MATCH [15]	All, advanced solid tumors	Master protocol Phase II, multicenter, open-label, nonrandomized Basket trial	New biopsy or recent biopsy of <6 months with no interim therapy sequencing assay for more than 4, 000 different variants in 143 genes	Pts with MA are assigned in one of predefined treatment cohorts	ORR	• Successful laboratory testing for 93% of pts • 18% of screened tumors was found to have a genetic mutation that matched the patient to	As of July 2017, 5963 tumor samples have been screened

Continued

Table 2. *Continued*

Study	Tumor	Study design	Biomarker	Methodology	End point	Identification of target and number of treated patients	Results and impact on outcome
						1 of the 30 treatment arms. • 998 pts have been assigned to treatment, of which 69% have enrolled (12% of screened population)	
Mypathway [34]	Advanced refractory solid tumor harboring MA in *HER2, EGFR, BRAF* or Hedgehog pathway	Master protocol Phase IIa, multicenter, non-randomized, multiple basket study	MP was not conducted as part of this study.	Pts are assigned to specific treatment cohorts based on the presence of a relevant target MA	Investigator-assessed ORR within each tumor-pathway cohort	NA, pts were only included if testing already carried out outside the clinical trial	Efficacy analysis population: 230 pts ORR: 23% within 14 different tumor types
SUMMIT [35]	Solid tumors harboring *HER2* and *HER3* mutations	Master protocol Multicohort basket study	MP was not conducted as part of the study, locally reported *HER2/3* mutations were confirmed centrally	Pts with *HER2*-mutant cohorts were enrolled into disease-specific cohorts and HER3 mutants into one cohort	Investigator-assessed ORR	NA	Total: 125 *HER2* mutant pts and 16 *HER3* mutant pts For *HER2* mutant tumors, primary end point was met only for breast cancer (ORR 32%) and not for lung, colorectal or bladder. No responses were observed in the *HER3* mutant cohort

aCGH, comparative genomic hybridization array; CNA, copy number alteration; DCR, disease control rate; FFPE, formalin-fixed, paraffin-embedded; FFS, failure-free survival; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; MA, molecular alteration; MP, molecular profile; NGS, next generation sequencing, ORR: overall response rate, PFS: progression free survival, Pts: patients.

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Table 3. Selected histology specific biomarker-driven approaches

Study	Tumor	Study design	Biomarker	Methodology	End point	Identification of target and number of treated patients	Results and impact on outcome
The BATTLE trial [16]	NSCLC	Master protocol Randomized phase II, single-center, open-label study	Fresh biopsy (FFPE) Testing of 11 prespecified biomarkers: PCR-based sequencing for mutations *(EGFR, KRAS, and BRAF)*, CNA by FISH *(EGFR, CCND1)*, and protein expression levels by IHC	Multiples arms: five biomarkers groups with different targeted therapies equal random assignment for 97 first pts, and adaptive randomization for next 158	DCR at 8 weeks	341 pts enrolled: 299 with adequate tissue for analysis (88%) 255 pts were randomized (75%)	Overall 8 weeks DCR: 46% Biomarker groups less predictive than individual biomarkers
SAFIR01 [29]	Metastatic breast cancer	Screening program	Fresh biopsy aCGH for preselected genes and Sanger sequencing for mutational hotspots on *PIK3CA* and *AKT1*	Screening: Based on the identified genomic alteration, pts were treated with targeted therapy if possible (within clinical trial or not)	Proportion of pts for whom a targeted therapy could be offered	423 pts included, biopsy obtained for 407 pts Targetable alteration in 195 (46%)	Therapy could be personalized in 55/423 pts (13%)
LUNG-MAP master protocol [27]	Advanced lung squamous cell carcinoma	Master protocol Phase II–III umbrella trial	Archival FFPE or fresh tumor biopsies FoundationOne NGS assay (Foundation Medicine) for mutations, amplifications, rearrangements (324 genes) and some IHC	Mutiple arms: Based on the molecular profile, each pt is enrolled in a sub-study with matched targeted therapy or in nonmatch substudy	ORR	1392 pts registered to the screening component 523 pts registered to a sub-study (37%)	First results for 3 biomarker driven cohorts (S1400B, S1400C and S1400D): ORR 4%–7% Cohorts closed due to futility at interim analysis S1400A (immunotherapy): 16% ORR Other sub-studies ongoing
The National Lung Matrix [23]	Advanced NSCLC	Master protocol Phase II umbrella trial	Prescreening of tumor biopsies through the Stratified Medicine Program 2 (take place in parallel with the patient receiving first line treatment): adaptable 28-gene NGS sequencing platform designed by Illumina covering the range of molecular abnormalities being targeted	Multiples arms (8 investigational medicinal products, within 21 distinct cohorts) Pts are allocated to the appropriate targeted therapy according to the molecular genotype of their cancer Bayesian adaptive design 'No actionable mutation arm' for patients without specific eligibility for one	ORR or PFS	As of July 2016: • 1664 pts tested • 1229 passed QC step (74%), 1098 pts with NGS results (66%) • 731 pts with aberration for MATRIX (44%) • 458 pts (28%) with MA and eligible (not registered) for MATRIX	As of 9 June 2017, 151 patients have been registered, 125 of these patients have received targeted treatments within the Lung Matrix trial. No results available per cohort. The Osimertinib cohort has been closed for recruitment.

Continued

Table 3. *Continued*

Study	Tumor	Study design	Biomarker	Methodology	End point	Identification of target and number of treated patients	Results and impact on outcome
				of the targeted genomic aberrations			
FOCUS4 [64]	Advanced colorectal cancer	Master protocol Phase II–III umbrella trial	FFPE block taken before commencement of standard chemotherapy Mutations of some preselected genes + some IHC, mRNA EREG	Multiple arms After induction chemotherapy, pts are enrolled in different cohorts on the basis of MA in the tumor, to test different targeted agents versus placebo or in a no-biomarker cohort testing standard capecitabine versus placebo as maintenance	PFS	NA	First results for 1 patient cohort (FOCUSD): Median PFS 3.48 months with placebo and 2.96 months with AZD8931: closed for futility

aCGH, comparative genomic hybridization array; CNA, copy number alteration; DCR, disease control rate; FFS, failure-free survival; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; MA, molecular alteration; MP, molecular profile; NGS, next generation sequencing; ORR, overall response rate; PFS, progression free survival; Pts, patients; QC, quality check.

includes both targetable alterations and genomic alterations that cannot be directly targeted but that lead to dysregulation of a pathway in which there are possible targets.

The percentage of patients that had an actionable genomic alteration identified through screening programs ranged from 46% to 63% [18, 20, 21, 29]. However, the number of patients who were finally treated with a matched targeted therapy was low: 13%, 16%, and 19% in SAFIR01 [29], IMPACT (first published report) [21], and MOSCATO 01 [20], respectively. This number increased to 27% in the most recent IMPACT publication [18], probably related to the extension of the screening panels. Different reasons may explain these low enrolment rate: tumor tissue issues, decline in the performance status or rapidly progressing disease, the absence of a targetable event, and the access to matched clinical trials or drugs. As IMPACT and the MOSCATO 01 were screening programs, patients were referred to enrolling clinical trials with obvious limitations in the treatment possibilities.

A way to partially solve these issues is to include the access to drugs into the clinical trial design. The NCI-MATCH basket trial pre-planned the access to some targeted compounds. However, only 12% of the patients were finally enrolled in the trial [22]. This low enrolling rate might be due to the low incidence of the targeted variants since only 18% of the screened tumors were found to have a genomic alteration that matched one of the 30 treatment arms. In contrast, in BATTLE and LUNG-MAP, two umbrella trials for NSCLC, 75% and 37% of the patients were included in one of the substudies, respectively [16, 27]. The number of treated patients is higher in these two last trials due to a preplanned access to matched targeted therapies. In addition, for the Battle trial, the molecular profile strategy was disease-specific and adapted to NSCLC, explaining the high prevalence of some of the investigated biomarkers.

Treatment efficacy in master protocols

Treatment selection based on DNA biomarkers has proved its efficiency: anti-HER2 therapies for *HER2* amplified breast cancer [30] and EGFR or pan-HER inhibitors for *EGFR* mutated NSCLC [31]. Pembrolizumab has been approved, independently of the tumor type, for microsatellite instability-high and mismatch repair deficient cancers [32] as well as for the first-line treatment of metastatic NSCLC with high PD-L1 expression [33].

Different end points are used in biomarker-driven trials. In MOSCATO 01 [20], the primary end point was the progression-free survival (PFS) ratio calculated for each patient, that must be >1.3 to define clinical benefit (PFS ratio =PFS on the molecular-profile selected therapy/PFS on prior therapy). The approach is judged efficient if it modifies the natural history of the disease and is associated with a longer PFS than the previous line of treatment. Thirty-three percent of patients treated with a targeted therapy had a PFS ratio >1.3. However, the number of patients who benefited from the personalized approach represented only 7% of the screened patients.

In IMPACT, the clinical outcomes of patients with molecular aberrations treated with matched therapy were compared with those of consecutive patients who were not treated with a matched therapy. They reported a better objective response rate (ORR) (11% versus 5%), a longer failure-free survival (3.4 versus 2.9 months), and a longer OS (8.4 versus 7.3 months) in the matched group [18]. The clinical benefit rate in the matched group,

Table 4. Ongoing biomarker-driven trials in squamous cell carcinoma of the head and neck

Study title	ClinicalTrials.gov identifier and status
Pan FGFR kinase inhibitor BGJ398 in treating patients with *FGFR1–3* translocated, mutated, or amplified recurrent head and neck cancer	NCT02706691 Not yet recruiting
Phase II study of tipifarnib in squamous head and neck cancer with *HRAS* mutations	NCT02383927 Recruiting
Copanlisib in association with cetuximab in patients with recurrent and/or metastatic head and neck squamous cell carcinomas harboring a *PI3KCA* mutation/amplification and/or a PTEN Loss	NCT02822482 Recruiting
SF1126 in recurrent or progressive SCCHN and mutations in *PIK3CA* gene and/or PI-3 kinase pathway genes	NCT02644122 Terminated (slow enrollment)
Korean Cancer Study Group: Translational blomarker Driven UMbrella Project for Head and Neck (TRIUMPH), Esophageal Squamous Cell Carcinoma- Part 1 (HNSCC)	NCT03292250 Recruiting

defined as the proportion of patients with either a stable disease lasting more than 6 months or a partial response or complete response, was 29% (111/381) when compared with 24% (56/238) in the nonmatched group. However, only 8% of the whole population finally experienced a clinical benefit. The use of nonoptimal targeted drugs or suboptimal dosages in phase I trials, and sometimes the level of evidence concerning the investigated biomarker(s) may explain the limited treatment efficacy observed.

In MyPathway basket trial [34], the ORR was 23% in 14 different tumor types, a clinically significant result for advanced refractory disease. In the SUMMIT trial [35], a basket trial studying neratinib in patients with a tumor harboring either *HER2* or *HER 3* mutations, the primary end point was reached only for breast cancer, and not for lung, bladder, and colorectal cancers, underlining the importance of the histology and the tissue of cancer origin. In BATTLE [16], the 8-week disease control rate and ORR were 46% and 4%, respectively. The first data of the ongoing Lung-MAP trial reported an ORR of 4%–7% for the first three biomarker-driven cohorts [27].

The SHIVA trial was the first randomized trial comparing a molecularly targeted therapy based on tumor molecular profiling versus conventional therapy for advanced cancer [17]. This study tested the overall strategy of a biomarker-driven treatment approach versus standard therapy. The trial did not meet its primary end point (PFS). Several reasons could explain this overall negative result. First, they used drugs that were marketed in France at that time and not necessarily the best in class to target the molecular alteration identified. Second, the experimental arm was also heterogeneous with multiple drugs and various tumor types. This could have blinded the benefit of some drugs in some specific cancer(s). The ongoing NCI-MPACT trial [15] is also a strategy trial. To avoid a negative trial linked with inadequate target modulation by the selected agents, all the targeted agents used in NCI-MPACT have been validated to engage their purported targets and have at least an established phase II dose.

Biomarker-driven studies for SCCHN

Only a few biomarker-driven trials are dedicated to SCCHN (Table 4). Some phase II trials are selecting patients upfront based on a rare specific genomic alteration [*HRas* proto-oncogene (*HRAS)* mutations or fibroblast growth factor receptor (*FGFR)* mutations/amplifications/translocations]. However, these trials offer only one potential therapeutic option for the very low percentage of patients harboring these rare genomic events. This results in a high rate of screening failure. There is another ongoing trial in Korea assessing personalized therapy for recurrent/metastatic SCCHN and esophageal cancer (NCT03292250) where patients are allocated to different treatment arms after first line platinum-based therapy according to molecular characterization.

Actionable or targetable genomic alterations in SCCHN

Next generation sequencing (NGS) technologies have identified potentially actionable/targetable genomic alterations in SCCHN [4, 9, 10]. Targetable genomic alterations in HPV-negative SCCHN include events in genes related to kinase growth factor family receptors or their downstream molecular pathways: *EGFR* (15%), *FGFR1–3* (14%), *HER2* (5%), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) (34%), and *HRAS* (5%). HPV-negative SCCHN has also potentially actionable cell cycle genomic alterations: *TP53* mutation (70%), cyclin D1 (*CCND1)* amplification (20%–30%), and *CDKN2A* inactivation (80%–90%). In HPV-positive OPC, where the oncoprotein E6 and E7 inactivate, respectively, p53 and Rb, *PIK3CA* amplifications/mutations are found in 56% whereas the other genomic alterations are rare.

The EORTC-1559-HNCG trial (UPSTREAM: Personalized STrategy for REcurrent And/or Metastatic SCCHN)

Our main objective was to design a biomarker-driven study dedicated to SCCHN patients. Below, we describe the overall study design as well as the different treatment cohorts.

EORTC-1559-HNCG design

The EORTC-1559-HNCG trial is a biomarker-driven umbrella trial that enrolls patients with recurrent/metastatic SCCHN,

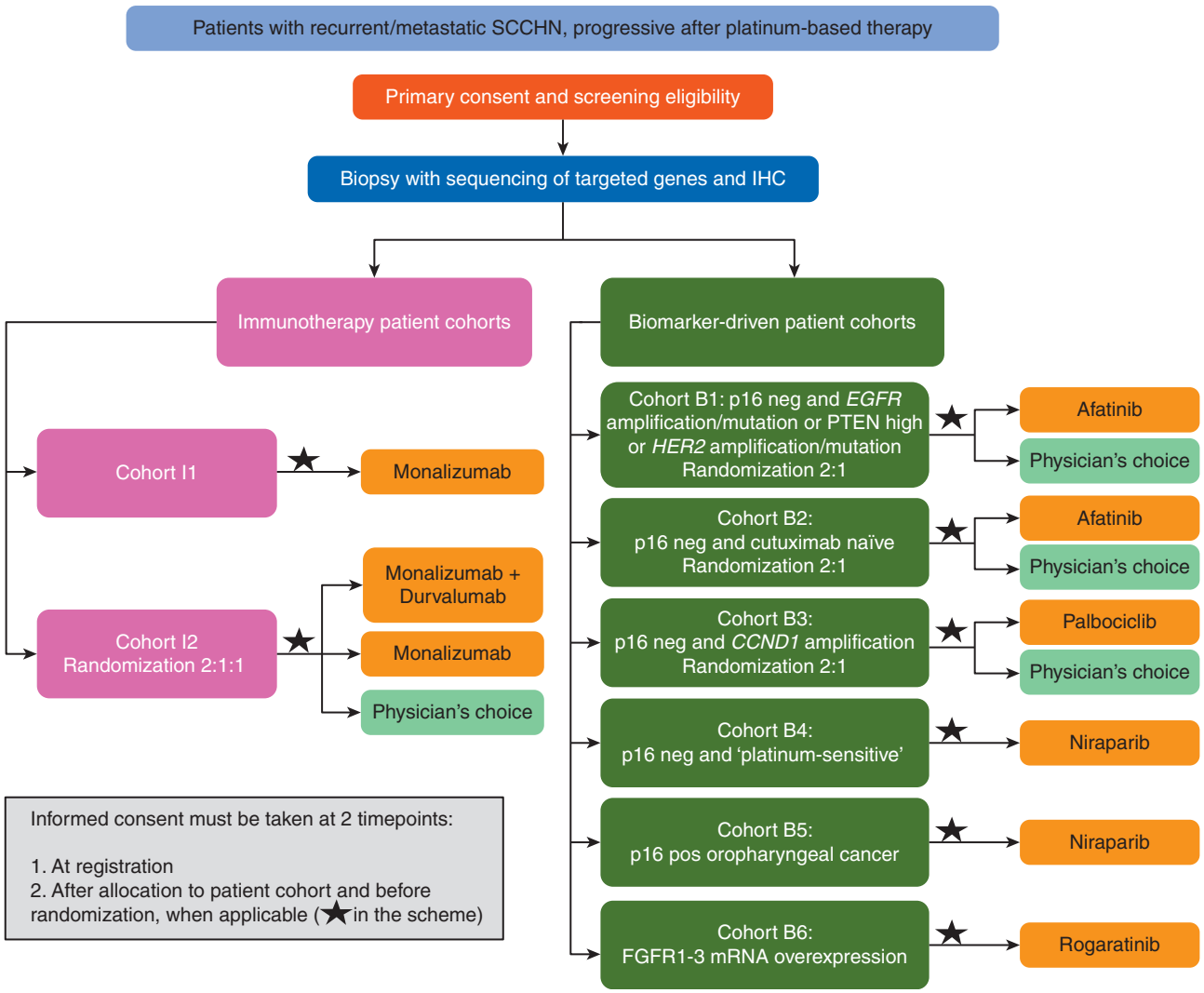

Figure 1. General design of the EORTC1559 umbrella trial.

progressing after first-line platinum-based chemotherapy. Each patient must undergo a fresh tumor biopsy. NGS is carried out to identify somatic mutations and copy number alterations with a custom panel that has been designed for the trial. This panel covers 13 oncogenes and tumor suppressor genes: *EGFR, HER2, TP53, PIK3CA, CCND1, NRAS, KRAS, HRAS, PTEN, FGFR1, FGFR2, FGFR3*, and *cMET*. The analysis also includes p16 (p16 positive =Histo-score ≥210) and PTEN (PTEN High =Histo-score >150) determined by IHC [36]. mRNA FGFR expression is evaluated by NGS. All these analyses are carried out centrally in an ISO 15189 certified laboratory (OncoDNA, Belgium).

Based on the molecular alterations identified, each patient is allocated to one of the cohorts. If the patient is not eligible for one of the biomarker-driven cohorts, he/she is included in one of the immunotherapy cohorts. The global design of the trial as well as the molecular rules for treatment allocation and prioritization are depicted in Figures 1 and 2.

The full protocol includes a core protocol and several addenda. The core protocol describes the overall study design, the objectives and end points, the inclusion/exclusion criteria, the study flow chart, the statistical hypotheses, the data analysis plan, and the biobanking processes. For each experimental treatment, there is one separate addendum that contains the confidential information related to the drug. The national health regulatory authorities, the ethical committee, and the investigators have access to the core protocol and all the addenda. The pharmaceutical companies have access to the core protocol but they can view and comment only the addendum/addenda concerning the cohort(s) for which they are supporting.

EORTC-1559-HNCG biomarker-driven and immunotherapy cohorts

Each patient cohort is designed as a phase II study with its own statistical hypothesis (Table 5). The primary end point is either ORR or PFS rate. Sample sizes vary from 32 to 76 patients across cohorts. The study can be amended to add other cohorts based on drug availabilities or other biomarker hypotheses.

Pan-human epidermal growth factor receptor (HER) inhibitor cohorts. EGFR mutations/amplifications are described in 15% of HPV-negative SCCHN and *HER2* is altered (mutation/amplification) in 5%.

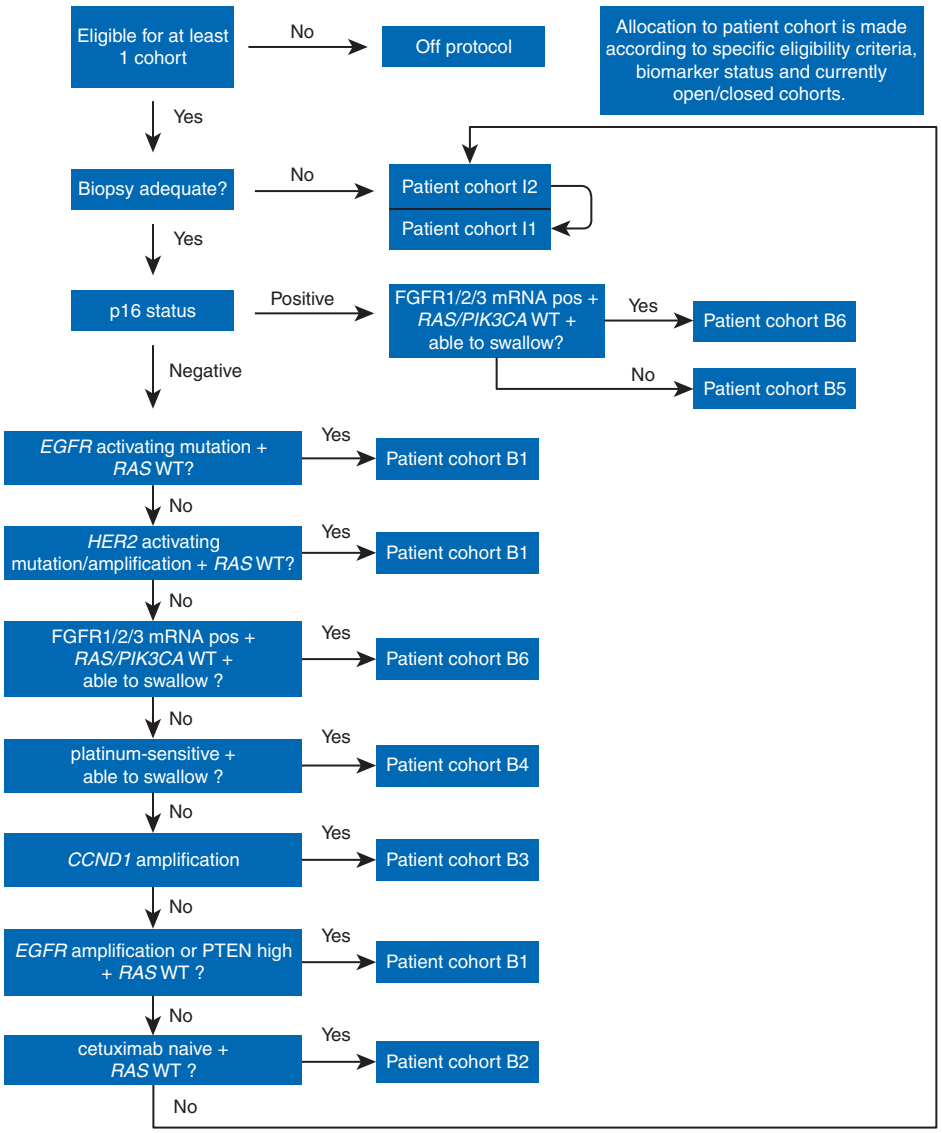

Figure 2. Prioritization algorithm for the allocation to different patient cohorts.

The ORR with cetuximab monotherapy is 13% [37]. In contrast to colon cancer where *RAS* mutations are predictive markers of resistance, *RAS* alterations are found in only 4% of HPV-negative SCCHN. Although *RAS* mutations might also play a role in cetuximab resistance in SCCHN [38], other mechanisms including activation of other HERs are involved [39, 40].

Pan-HER inhibitors target all the dimers forms by HER family and have the potential to overcome anti-EGFR therapy resistance caused by cross-talk between EGFR and the other HERs. In unselected SCCHN patients who progress after platinum therapy, afatinib, an irreversible pan-HER inhibitor, improves PFS compared with methotrexate: median PFS 2.7 versus 1.6 months [41]. However, afatinib does not increase OS. Biomarkers analyses were carried out within this trial [36]. Median PFS favored afatinib in patients with p16-negative, *EGFR*-amplified (defined as $\geq$50% of cells with $\geq$4 copies, or $\geq$1 cell with $\geq$8 copies), HER3-low (defined as *H*-score $\leq$50), and PTEN-high (defined as *H*-score >150) tumors. In the MCC15780 trial where 38 SCCHN patients were treated with cetuximab [42], PFS was also significantly increased in PTEN-high tumors compared with PTEN-low tumors [43]. The fact that afatinib seemed to be more active in case of HER3-low and PTEN-high disease suggests that pan-HER inhibitors could be more active when the PI3K pathway is not or less activated. Cetuximab-naïve patients with p16 negative tumor had also a significant benefit from afatinib (ORR: 27%).

We designed two biomarker-driven cohorts in the EORTC-1559 trial where the patients are randomized between afatinib or investigator's choice. The first cohort includes patients with p16 negative SCCHN harboring either an *EGFR* mutation/amplification or *HER2* mutation/amplification or PTEN high (*H*-score >150). We did not include patients with HER3 low disease as this IHC is not always reproducible [44]. The second cohort includes cetuximab-naïve SCCHN patients with p16-negative tumor. SCCHN with any *RAS* mutations are excluded [38].

Table 5. Different patient cohorts of EORTC HNCG 1559 trial

Patient cohort	Biomarker(s)	Targeted drug/IO	Design	Sample size (max)	Statistical hypothesis
Biomarker-driven patient cohorts					
B1[a]	p16 negative and *EGFR* amplification/mutation or PTEN high or *HER2* amplification/mutation	Afatinib	Phase II, randomized, open-label, multicenter study Simon 2 Stage design	55	H0: PFSR at 16 weeks=20% H1: PFSR at 16 weeks=40%
B2[a]	p16 negative and cetuximab naïve	Afatinib	Phase II, randomized, open-label, multicenter study Simon 2 Stage design	55	H0: PFSR at 16 weeks=20% H1: PFSR at 16 weeks=40%
B3	p16 negative and *CCND1* amplification	Palbociclib	Phase II, randomized, open-label, multicenter study Simon 2 Stage design	55	H0: PFSR at 16 weeks=20% H1: PFSR at 16 weeks=40%
B4	p16 negative and 'platinum-sensitive'	Niraparib	Phase II, single arm, proof-of-concept, multicenter study Simon 2 Stage design	32	H0: ORR over first 16 weeks=5% H1: ORR over first 16 weeks=20%
B5	p16 positive OPC	Niraparib	Phase II, single arm, proof-of-concept, multicenter study Simon 2 stage design	32	H0: ORR over first 16 weeks=5% H1: ORR over first 16 weeks=20%
B6[b]	FGFR1/2/3 mRNA overexpression	Rogaratinib	Phase II, single arm, proof-of-concept, multicenter study Simon 2 stage design	20	H0: ORR over first 16 weeks=5% H1: ORR over first 16 weeks=25%
Immunotherapy cohorts					
I1	NA	Monalizumab	Phase II, single arm, proof-of-concept, multicenter study Single stage A'Hern design	40	H0: ORR over first 16 weeks=3% H1: ORR over first 16 weeks=15%
I2	NA	Monalizumab +durvalumab	Phase II, randomized, open-label, multicenter study Simon 2 stage design	76	H0: ORR over first 16 weeks=3% H1: ORR over first 16 weeks=15%

[a]Patients included in the afatinib arms should not have activating mutation in *RAS*.
[b]Patients included in the Rogaratinib arm should not have activating mutation in *RAS* or *PIK3CA*.
ORR, overall response rate; PFSR, progression-free survival rate.

FGFR inhibitor cohorts. FGFRs can activate the RAS-MAPK, PI3K, STAT, and PLCγ pathways [45]. *FGFR1* mutation/amplification are found in 5%–10% of HPV-negative SCCHN, while *FGFR3* mutations are more frequent in HPV-induced OPC (1%–12%). Genetic alterations of *FGFR2* are observed in only 2%–4%.

Erdafitinib, a pan-FGFR inhibitor, induced ORR in 24%–35% of patients with metastatic urothelial cancer harboring *FGFR* alterations (including activating mutations and translocations) [46]. Twenty-four percent of patients with urothelial cancer overexpressing FGFR1-3 mRNA achieved ORR with Rogaratinib, another pan FGFR inhibitor [47]. Partial responses were also observed in some patients with squamous cell lung cancer, SCCHN, and adenoid cystic carcinoma [48]. Interestingly, some responding patients had elevated tumor FGFR3 mRNA levels without corresponding genomic alterations. The prevalence of FGFR1-3 mRNA positivity among 46 SCCHN patients was 56.5% [49].

We will investigate Rogaratinib in cases of high FGFR mRNA levels assessed by NGS.

Cell cycle inhibitor cohort. The vast majority of HPV-negative SCCHN harbors genetic alterations (*TP53* mutations, *CCND1* amplification, and p16 inactivation) that enable them to circumvent the mitotic checkpoints through aberrant cyclin-dependent kinase (CDK) activation. Since p16 inactivates CDK4/6 whereas cyclin D1 activates CDK4/6, there is a rationale to test CDK4/6

inhibitors in patients with p16 negative and *CCND1*-amplified SCCHN. Palbociclib in combination with cetuximab has been investigated in recurrent SCCHN with promising preliminary results (ORR: 35%) [50]. However, palbociclib monotherapy has not been investigated in SCCHN.

We will investigate palbociclib in patients with p16 negative tumors harboring *CCND1* amplification.

Poly-ADP ribose polymerase inhibitor cohorts. DNA repair deficiency increases sensitivity to platinum-based chemotherapy and poly-ADP ribose polymerase (PARP) inhibitors [51]. A comprehensive analysis for homologous recombination deficiency (HRD) was carried out, and HRD was associated with ovarian, lung, SCCHN, and bladder cancer. Preclinical studies have shown that HPV-positive SCCHN have DNA double strand repair defects responsible for increased sensitivity to the PARP inhibitor veliparib [52]. These data support the two patient cohorts that will investigate niraparib, another PARP-inhibitor, in p16-positive OPC and in platinum-sensitive p16 negative SCCHN.

Immunotherapy cohorts. PD-1/PD-L1 blockers have activity in SCCHN but the 2-year's OS rate is still low: 16.9% [53]. Therefore, other immunotherapy approaches have to be investigated.

HLA-E is a nonclassical major histocompatibility complex molecule that constitutes a way for cancer cells to escape immune surveillance. HLA-E is highly expressed in 70% of SCCHN [54]. HLA-E binds to NKG2A receptor on NK cells and T-lymphocytes to inhibit the cytotoxic functions of CD8+ T lymphocytes and NK cells. Monalizumab is a human IgG4 antibody targeting the NKG2A receptor. In the first immunotherapy cohort, patients will receive monalizumab monotherapy. In the second immunotherapy cohort, patients will be randomized to receive the combination of durvalumab and monalizumab versus monalizumab monotherapy versus physician's choice.

EORTC1559 feasibility

The trial is open for inclusion since December 2017. On 19 July 2018, 19 sites are open in 3 countries. Sixty-four patients have been screened, 24 included in one of the biomarkers cohorts, and 23 in one of the immunotherapy cohorts. The turnaround time between the biopsy and the molecular diagnosis provided by the central laboratory is 10 calendar days.

Discussion

The EORTC-1559-HNCG trial is the first European international umbrella trial assessing a personalized treatment strategy for patients with recurrent/metastatic SCCHN. We hypothesize that this approach can improve patients' outcome.

The trial design has different strong points: one single protocol with pre-planned access to matched targeted therapies, one fresh tumor biopsy to deal with tumor evolution over time, an ISO-certified central laboratory, well-defined biomarker hypotheses, and the possibility to have a never-ending protocol with the opportunity of adding new cohorts.

Besides the inherent complexity of such trials, numerous logistic and scientific challenges were encountered when designing this protocol.

Although the pharmaceutical companies accepted the concept of having only one protocol including the different compounds, complex negotiations were crucial to successfully achieve that all stakeholders agreed (i) to standardize the processes, (ii) to accept the predefined protocol structure, (iii) to use the central biomarker laboratory, (iv) to match the company interests with the academic wishes, and (v) to align all the companies on the same protocol wording in particular for the inclusion/exclusion criteria. The protocol was submitted in four different countries (Belgium, France, Italy, and UK) and will be submitted in Germany to both competent authorities (CA) and applicable ethics committees (EC). Overall, the study was well received by the CA and EC without major comments on the study design. The main question received from EC was concerning the criteria to allocate patients to the different cohorts. Regarding the regulatory strategy, having all those cohorts in only one study simplifies the submission process, as it requires only one initial clinical trial application to each CA and one initial request of opinion to each EC. Also, each amendment can group modifications concerning more than one cohort at the same time. If we had considered each cohort as one trial, different submissions would have been necessary, increasing the regulatory workload and probably time for activation. As separate trials, the advantage would have been that the current cohorts could be opened/closed independently across the countries without the need of a main protocol amendment. In addition, the liaison with the stakeholders would be easier, as the number of stakeholders per trial would be significantly reduced.

The new European clinical trials regulation [55] fully in application next year might bring a novel perspective for studies with a complex design. Multiple member states will participate on the coordinated assessment of some sections of the dossier, ensuring that consolidated communication reaches the applicant. This may reduce the volume of correspondence and facilitate the management of any protocol modifications if they are required.

Several challenges remain. Optimal management of country-specific documents adaptation and effective communication with the stakeholders might be the key to ensure fulfillment of adequate deadlines and quick activation of new cohorts to follow the fast advancing head and neck cancer research field.

At the scientific level, the study is still missing some treatment arms that target important genetic aberrations. *PIK3CA* alterations occur in 16%–34% of HPV-negative patients and in up to 56% of HPV-positive patients. Patient-derived SCCHN tumor xenografts with *PIK3CA* activating mutations are sensitive to mTOR/PI3K inhibitors [56] and, in the BERIL-1 trial, buparlisib improved OS when added to paclitaxel [57]. Among other interesting targets, there is a scientific rationale to test Farnesyl transferase inhibitors in the 5% of SCCHN harboring *HRAS* mutations or WEE1 inhibitors in *TP53* mutated tumors.

In the current design, immunotherapy cohorts are not linked to biomarker(s). Among others, HPV-positivity, PD-L1 overexpression, in-frame, or frameshift alterations of specific tumor suppressor genes, and mutational burden are potential biomarkers that have been associated with a higher efficacy of immunotherapy in SCCHN [7, 8, 58]. However, these predictive

markers are far to be optimal. Umbrella trials represent an ideal platform to further investigate the predictive value of immune biomarkers.

We cannot deny that tumor heterogeneity that can cause treatment resistance is not addressed by the use of targeted compounds in monotherapy. Therefore, we also collect whole blood, plasma as well as tumor biopsies for translational research. Analyzing these biologic samples will give us more insight on the genetic landscape of recurrent/metastatic SCCHN, which may lead to the discovery of new therapeutic targets, and may help to investigate more precisely the utility of liquid biopsy. Translational research will also provide information regarding drug resistance mechanisms and will help us to develop new combination treatments that are able to tackle them.

A finding of biomarker-driven studies is the low number of patients who benefit from this approach. This suggests that for heterogeneous cancers with multiple potential oncogenic drivers, biomarkers assessed only at the DNA level may not predict drug responses reliably. The signification of some genomic alterations can vary from one cancer histology to another. Therefore, for further developments, we will have to take into account several others parameters such as the phenotype (e.g. gene expression/proteomic profiles) and the tissue of cancer origin [59].

In conclusion, precision medicine remains a major challenge for the medical community. Large efforts are needed to optimize the study designs, the theranostic tools, and the trial logistics. Designing biomarker-driven studies requires close collaboration with country CA, EC, and pharmaceutical companies to reduce the administrative burden and facilitate the processes linked with the design and conduct of such clinical trials.

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Disclosure

J-PM is a member of the advisory board of MSD (uncompensated) and INNATE; CLT has been part of advisory boards of MSD, BMS, Merck Serono, Roche, Amgen, Novartis, Nonobiotix; JG has been part of advisory boards for AstraZeneca, Bristol-Myers Squibb, Innate Pharma, and Merck KGaA and has received grants for research from GSK, Bristol-Myers Squibb, Chugai, and Merck KGaA; LL has served as consultant/adviser and/or give lectures for Astrazeneca, Bayer, BMS, Boehringer Ingelheim, Debiopharm, Eisai, Merk-Serono, MSD, Novartis, Roche and Sobi. She has received research funds from AstraZeneca, Boehringer Ingelheim, Eisai, Merck-Serono, MSD, Novartis and Roche. She received travel coverage for medical meetings from Bayer, BMS, Debiopharm, Merck-Serono, MSD and Sobi; JFL is an employee of OncoDNA; ES-B is a member of advisory board of BMS; AK has served as an adviser for PUMA Biotechnology and Avvinity/Centuari Therapeutics Limited. He has received research grants from AstraZeneca and PUMA Biotechnology and has also received honoraria from Merck, BMS and MSD as an invited speaker.

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