RESEARCH ARTICLE

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Two by Two Factorial Design using Metformin and Curcumin for Second Primary Head and Neck Cancer Prevention Trial

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Abstract

Objective: The 2x2 factorial design is an effective method that allows for multiple comparisons, especially in the context of interactions between different interventions, without substantially increasing the required sample size. In view of the considerable preclinical evidence for Curcumin and Metformin in preventing the development and progression of head and neck squamous cell carcinoma (HNSCC), this study describes the protocol of the clinical trial towards applying the drug combination in prevention of second primary tumors. **Methods:** We have applied the trial design to a large phase IIB/III double-blind, multi-centric, placebo-controlled, randomized clinical trial to determine the safety and efficacy of Metformin and Curcumin in the prevention of second primary tumours (SPT) of the aerodigestive tract following treatment of HNSCC (n=1,500) [Clinical Registry of India, CTRI/2018/03/012274]. Patients recruited in this trial will receive Metformin (with placebo), Curcumin (with placebo), Metformin, and Curcumin or placebo alone for a period of 36 months. The primary endpoint of this trial is the development of SPT, while the secondary endpoints are toxicities associated with the agents, incidence of recurrence, and identifying potential biomarkers. In this article, we discuss the 2x2 factorial design and how it applies to the head and neck cancer chemoprevention trial. **Conclusion:** 2x2 factorial design is an effective trial design for chemoprevention clinical trials where the effectiveness of multiple interventions needs to be tested parallelly.

Keywords: Head and neck squamous cell carcinoma- second primary tumour- curcumin- metformin

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Introduction

Head and neck squamous cell carcinoma (HNSCC) continues to be a significant cause of morbidity and mortality, especially in the Asia-Pacific region [1]. Despite advances in treatment, the overall survival of these patients has remained stagnant [2]. One of the principal reasons behind this is the high rate of second primary tumours (SPT) of the upper aerodigestive tract, with the annual and cumulative risks of SPT in these patients being 2-4% and 10-35% respectively [3-5]. With limitations in the available treatment modalities, the mortality of SPT is higher than in primary tumors [6]. The high incidence of SPT is attributed to field cancerization [7-9], with a clear distinction between SPTs (genetically unrelated, distinct tumours in the same field) and second-field tumours (genetically similar tumours in the same field). As Kuriakose and Sharan, [10] reported in their previous work, attributes of HNSCC that make it amenable to chemo preventive therapy are the strong association with etiological factors like tobacco and alcohol, presence of potentially malignant lesions like leukoplakia, a well-defined model for progression and the easy accessibility of the upper aero-digestive tract for clinical monitoring.

Chemoprevention in HNSCC has been studied previously, however, the issues with these agents have been toxicity and lack of durability of response, with none of them being recommended for routine use [11-13] (Figure 1). Among the agents, the phytopolylphenol pigment, Curcumin, an active ingredient isolated from Curcuma longa, has demonstrated chemo-preventive effect in a prospective, randomized clinical trial with Curcumin in patients with oral potentially malignant lesions [14]. Metformin, a drug in the family of biguanides, is shown to have anticancer properties; an observational cohort study reported that Metformin users are at a lower risk of cancer as compared with Type 2 diabetics on other treatments [15]. Pre-clinical studies by our group in a 4NQO carcinogenesis model have indicated a synergistic benefit of the use of two known chemo-preventive drugs, Curcumin and Metformin, in oral cancer [16]. Although known adverse event profiles and drug metabolism studies have indicated that Curcumin and Metformin are not expected to be overlapping, with no expected major adverse events, the combination has not been assessed in chemoprevention.

The primary objective of the SPT Trial; a randomized, prospective phase IIb/III, placebo-controlled, double-blind multi-centric clinical trial. [Clinical Registry of India, CTRI/2018/03/012274], is to determine whether the use of Curcumin and Metformin therapy in combination helps in reducing the incidence of SPTs among patients with HNSCC treated with curative intent. A two-by-two factorial design was adopted to investigate these agents individually and in combination. The study has four intervention arms; Metformin alone, Curcumin alone, a combination of Curcumin and Metformin, and a placebo. Secondary objectives intended to compare the incidence of tumor recurrence, determine the efficacy of Curcumin/Metformin towards regression of existing oral premalignant lesions (OPML) and reduction in the occurrence of new OPML, and finally to assess the adverse effect of long-term use of Curcumin and Metformin.

In this article, we discuss the rationale and design of a Phase III randomized double-blind control trial to determine the efficacy of Curcumin and Metformin to reduce the incidence of SPTs of the aero-digestive tract in HNSCC treated with curative intent.

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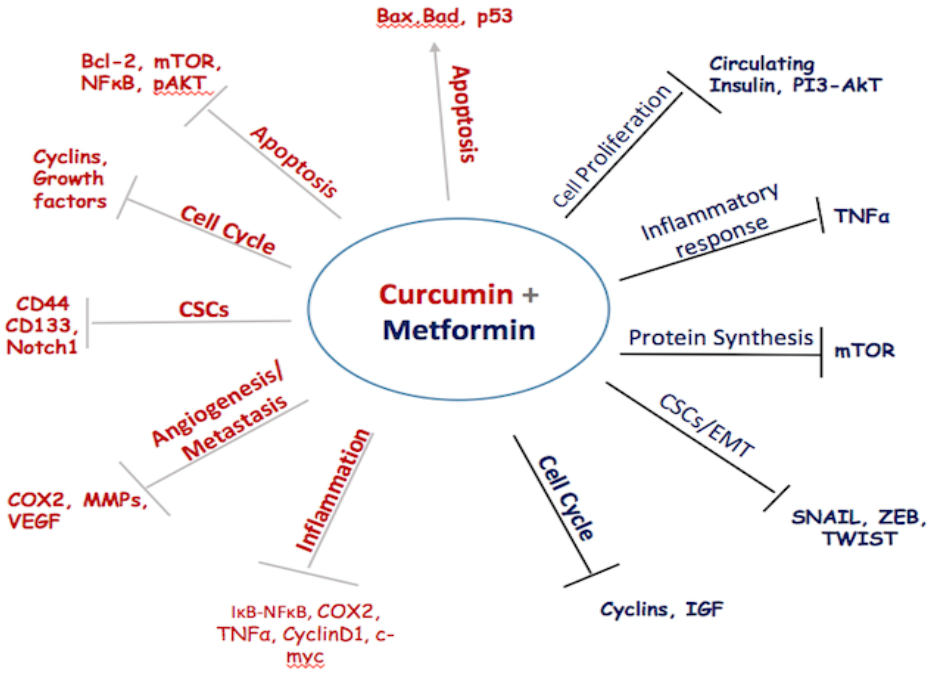

Figure 1. Combined Anti-Cancer Mechanisms of Curcumin and Metformin The Figure Depicts the Pathways that Curcumin and Metformin Target in the Cancer Cells.

determine the efficacy of Curcumin and Metformin to reduce the incidence of SPTs of the aero-digestive tract in HNSCC treated with curative intent.

Materials and Methods

Methods

Participants, Intervention, and outcomes

Study Setting

The subjects for this study will be recruited from 18 academic hospitals spread across India. The details of the participating centers are enlisted as annexure.

Eligibility Criteria

Preliminary eligibility will be assessed for patients in the age group between 18 and 80 years (with an anticipated lifespan of at least five years) and a history of Stage I to III (T1-3, N0-1, M0) HNSCC (excluding that of paranasal sinuses and nasopharynx) treated with curative-intent 3-12 months before consent. The study includes patients with early-stage disease to offset the effect of a higher rate of recurrence in advanced stages. Pathologic stage, if available will be used as inclusion criteria. For the larynx and oropharynx cases treated primarily by non-surgical modalities, clinical staging will be considered. Patients who are deemed free of disease, have recovered from initial treatment-related sequelae, have a Karnofsky Performance Score ≥80, have normal blood counts and liver and renal function are considered eligible. Women of childbearing age will need to use contraception for at least a year after completion of the course of chemoprevention.

Intervention

Pre-randomization Screening Assessment

Potentially eligible patients will undergo baseline head and neck and general physical examination, direct laryngoscopy, urine, and hematological investigations to confirm eligibility criteria. Patients will be screened based on their medical history, drug history, physical examination, blood screening, and HbA1c. The study will be conducted with a run-in phase, where subjects will enter into an active run-in period for 2 weeks, wherein the patient will receive 1g of Metformin and 1.2 g of Curcumin for 2 weeks and reassessed. Patients who have no adverse reactions and remain compliant with medication and follow-ups will be randomized into the trial. Based on previous phase I data, toxicity is not anticipated at this dose.

Baseline clinic visit and randomization

At the baseline visit, written informed consent will be obtained from all eligible patients. A randomization schedule will be established for each participating center separately using computer-generated random numbers. A set of randomization codes and the corresponding labeled drug kits will be dispatched to each center in advance. For eligible patients, a randomization request will be sent to the centralized project office, where an unblinded biostatistician will review the completeness of the request and mail the appropriate treatment box number to the center, which will dispense the corresponding drug kit to the patient.

Intervention groups

The 2x2 factorial study design has been utilized for this trial (Table 1). The intervention arms will receive 1.2g of Curcumin (2x 600mg tablets), 1g of Metformin (2x500mg tablets), or a combination of both daily for 36 months. Metformin will be dispensed as tablets consisting of 500mg of active medication and Curcumin

Table 1. 2x2 Factorial Design. Patient distribution across four treatment arms is 1:1:1:1. The unbiased randomization is ascertained using SAS version 8.2.A block randomization scheme will be used to ensure balance of treatment and placebo groups at each center

Drugs		Curcumin	
		Yes	No
Metformin	Yes	Arm CM : Curcumin (2x 600mg tablets) or 1gm of Metformin (2x 500mg tablets)	Arm M : Metformin (2x 500mg tablets) + Placebo
	No	Arm C : Curcumin (2x 600mg tablets) + Placebo	: Arm P Placebo + Placebo

will be dispensed as soft gel capsules of 600 mg each. The placebo arm includes capsules of a similar shape and color as the drug to ensure blinding and will be dispensed similarly to the treatment arm. The drugs administered/scheduled for each of the arms are i) Arm P: Placebo (Curcumin placebo: twice daily (2*600mg); Metformin placebo: twice daily (2*500mg)) ii) Arm C: Curcumin (Curcumin: twice daily (2*600mg); Metformin placebo: twice daily (2*500mg)) iii) Arm M: Metformin (Curcumin placebo: twice daily (2*600mg); Metformin: twice daily (2*500mg)) and iv) Arm CM: Combination (Curcumin: twice daily (2*600mg); Metformin (twice daily (2*500mg)). Protocols have been put in place to ensure the safe storing of the medication to ensure the safety and efficacy of active ingredients. Compliance logs are being used to ensure patients have been on the medication prescribed; all previous bottles are to be returned before the dispensation of fresh medication wherein the number of remaining pills will be counted as a reflection of patient compliance. Patient specimens (blood, saliva, surgical tissue, and buccal smear) will be collected for future studies during each visit after written informed consent.

Adverse event evaluation

Adverse Events (AE) will be graded for severity and relationship to the study product and will be evaluated using NCI Common Adverse Event Reporting forms (version 5.0) (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf). Each adverse event will be graded as per NCI definitions to Grades I-V. A data safety monitoring board (DSMB) has been established to periodically review the progress and safety of the trial and recommend necessary changes.

Withdrawal criteria

Patients who fail to comply with protocol, are lost to follow-up, suffer from severe adverse events, or withdraw consent will be withdrawn, however, an intent-to-treat analysis will be performed. Patients who develop recurrence or a second primary tumor will be considered to have achieved the end of the study and will be removed from the study after performing a biopsy, contrast CT scan, and other relevant tests as per the principal investigator's discretion.

Follow-up visits

Patients will be followed up every 3 months for 3 years (Figure 2). The Assessment as per the schedule of events (Table 2) will be performed and analyzed to determine whether therapeutic intervention may modify the development of a second primary tumor and if it has a positive impact on the recurrence of the index primary tumor. Any oral premalignant lesion within the head and neck region will be identified and the size recorded in the Case Record Form (CRF). These lesions will be histologically characterized by biopsy before the enrollment of a patient. Any alteration in size (increase or decrease) or clinical appearance during the follow-up visit will be noted and if required biopsy will be performed. Subjects who develop new premalignant lesions will stay on the trial. The Safety profile of the combination therapy will be assessed by screening the subjects at each visit or when they report for signs or symptoms

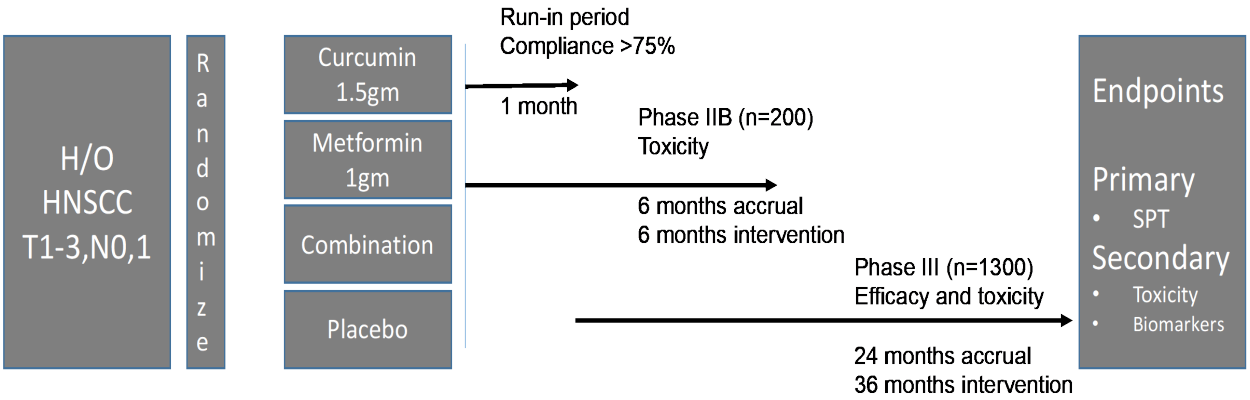

Figure 2. Schematic Representation of the Study. The figure depicts a flowchart of the events in the trial. The patients are placed in a run-in period for a duration of 2 weeks before randomization to rule out any acute side effects. The patients will be recruited for the Phase IIB and subsequently in the Phase III of the trial. The primary and secondary endpoints of the trial are indicated.

Table 2. Patient Follow-up Schedule. Contrast CT scan will be performed at the end of the study. In addition to the planned CT scan, imaging will be done as indicated based on the clinical examination and chest X-ray findings. All intervention medication will be stopped 48 hours before CT scan and restarted after Serum Creatinine measurement carried out 3 days after CT scan. Patient follow-up window period of ±7 days will be accepted from actual follow-up visit. Blood tests are performed once in 3 months in the initial 1 year to rule out any chronic toxicity and to decide the dose modification if required.

Evaluation/ Procedure	Baseline	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Month 27	Month 30	Month 33	Month 36
Medical History	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical/Laryngoscopy Examination	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Tests	X	X	X	X	X		X		X		X		X
Tobacco and alcohol cessation counseling	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X
X-ray chest	X		X		X		X		X		X		X
CT examination of neck and chest	X												X

of any kind of adverse events and recorded in the CRF. Specifically, patients will be assessed for the presence of GI symptoms that could suggest GI ulceration such as epigastric pain, cramps, diarrhea, melena, and hemoptysis. Scheduled blood tests will be reviewed for evidence of potential drug toxicity. The compliance of long-term use of Curcumin and Metformin will be analyzed through clinical monitoring and blood investigations.

Outcomes

The main outcome will be the development of a second primary tumour and/or recurrence. Warren and Gates' criteria [17] have been considered standard criteria to distinguish between SPTs and recurrences. However, given that in the upper aerodigestive tract proving one tumor does not represent a recurrence of the primary tumor is difficult, additional criteria have been used. They include a distance of at least 2 cm between the first tumor and the next [18]. A time period of three years between the appearance of tumors to exclude recurrence and confirm SPTs has been described [19]. Further in this study, we have considered SPTs as both SPTs and second-field tumors [20]. Radiological criteria like topography, lesion number, range of calcification, and temporal changes In the lesion size and morphology will be utilized for distinguishing second primary tumors and metastatic disease [21, 5].

Sample size

Considering a cumulative incidence of SPTs in the control group of 12% and a cumulative incidence in the combination therapy group of 7% with a duration of follow-up of 5 years with an 80% power and 5% level of significance, the number of samples required in each arm will be 340 patients. When accounting for a drop-out rate of 10%, the number required in each arm would be 374, with a total sample size of 1496 patients. With the 2x2 design, the calculation takes into consideration multiple comparisons in addition to the traditional 5% level. Typically, there are 3 effects estimated in a 2x2 design: the main effects of the two interventions and the interaction effect; as a result, we divide the overall p-value (5%) by 3 (number of proposed comparisons) for the sample size calculation to be sufficiently powered for these multiple comparisons (Bonferroni correction). Considering the above recommendations, we have arrived at a total sample size estimate of 1500 subjects.

Assessment of intervention

The patients will be randomized after evaluating eligibility criteria and obtaining informed consent. A randomization list for each study center will be generated using SAS version 8.2. This will be provided to the study pharmacist at each of the patient accrual centers. A block randomization scheme will be used to ensure a balance of treatment and placebo groups at each center. Simple randomization will be performed to randomize the patients. Based on the randomization list, the drugs will be dispensed with matching randomization numbers from the outpatient pharmacy by the pharmacist. Both the patients and investigators will be blinded to the treatment assignment. Randomization numbers and dates will be recorded in the patient records and CRF.

Data collection, management, analysis, and monitoring

The project manager at the coordinating center will coordinate the data capture and analysis. The Clinical Research Coordinator (CRC) initially captures all the patient data identified by study number in Case-Report Forms (CRFs), which include the center codes. Patient identifiers reside only in the medical charts. The CRCs will track and record data of each participant entering the run-in period, including lab results at their respective centers. The CRC is also responsible for digitizing the data (REDCap software) from CRFs under the supervision of the Project Manager and study statistician. The statistician will conduct random checks of data entry for quality checks Every practical effort will be made to maximize study participation to prevent dropouts. The magnitude of missing data is not expected to be extensive. However, the

pattern of missing data will be characterized and estimated using regression methods.

Results

Statistical considerations

The primary endpoint and major secondary endpoints analysis will be the incidence of SPTs and recurrences in the treatment and placebo arms after 36 months of interventions. Phase IIb analysis will be carried out after the completion of 1-year follow-up of the first 200 subjects. The Safety of the study drugs will be assessed based on clinical examination and laboratory investigations. If >50% of patients in the intervention arm show toxicity based on the NCI Common Toxicity Criteria, the dose of Metformin will be reduced to 500 mg. We intend to use a primary intent-to-treat analysis. Secondary analyses to determine the proportion of responders will be done using logistic regression techniques. This will allow us to relate the probability of clinical response to treatment with consideration to other categorical or continuous variables, such as sex, age, use of tobacco and alcohol, tumor stage, subsite, and biomarkers. Models fitting these covariates to the incidence of SPTs will be assessed via multiple-regression techniques. For the latter, the multi-collinearity of analyses will be minimized by identifying sets of covariates that correlate highly with one another (r>0.5) and choosing one variable to represent the set. Analyses of change in biomarker endpoints will be carried out by t-test, in a linear mixed model.

Current Status of SPT Trial

The patient recruitment under the SPT trial was initiated in August 2018 with 5 collaborating sites. An additional 13 centers were later included to aid in patient recruitment. As of 31 January 2023, 705 patients were randomized from a total of 854 patients screened for the study. The active patients in the trial currently are 508, with 80 patients having completed their 36 monthly follow-ups. While 48 patients discontinued the study due to various reasons, 8 patients developed unrelated serious adverse events. A total of 61 patients attained the end of the study due to recurrence, and second primary tumor development. An interim analysis of the trial is currently ongoing.

Discussion

Head and neck squamous cell carcinoma are one of the most common cancers in Asia-Pacific Region due to the rampant use of tobacco products, betel, and areca nut. Historically, loco-regional failure was a major factor affecting survival in HNSCC patients [22]. However, with improvements in surgical techniques, reconstruction, and advances in radiotherapy, there is a trend towards improvement of loco-regional control following treatment of index primary tumor, putting patients at a higher risk of developing SPTs. Therefore, strategies for the prevention of SPTs require urgent attention. Proof of concept for chemoprevention as an effective strategy has been established through studies with vitamin A and its analogs [10]. However, significant toxicities with long-term use pre-empt its routine use in clinical practice. In this study we describe the trial design towards assessing the efficacy of Curcumin and Metformin in chemoprevention of SPT in HNSCC.

Our trial offers a unique opportunity to potentially address these issues concerning SPTs in HNSCC. The primary advantage of the trial is the factorial study design, which allows for the testing of multiple treatment arms simultaneously, thereby ensuring optimization of time, expense and participant recruitment. The gold standard for defining new therapies is a randomized control trial (RCT). However, given that RCT requires considerable resources, time, and a larger sample size, a 2 x 2 factorial design provides an opportunity to optimize trial design efficiency. In lieu of conducting separate studies for each intervention, a factorial design allows for the investigation of multiple factors simultaneously. Further, this is a cost-effective approach facilitating the investigations with optimal patients in each arm. The gold standard for defining new therapies is a randomized control trial (RCT). However, given that RCT requires considerable resources, time, and a larger sample size, a 2 x 2 factorial design provides an opportunity to optimize trial design efficiency. The 2*2 design, specifically, allows an assessment of the interventions individually in addition to assessing their potential interactions that reflect in either a synergistic or an antagonistic effect when two treatments are administered together. This information is valuable for understanding the combined impact of multiple interventions, their safety and in this study, the efficacy in prevention of second primary tumors in HNSCC. The main concern with a 2 x 2 design is the assumption of non-interaction between therapies. As reported by Freidlin and Korn, [23] even if the oncological outcomes may differ the overlapping toxicities cannot be ruled out. In this study, an attempt is made to analyze the results of each arm individually to overcome the potential flaws of this design.

In this study, Curcumin and Metformin are being explored for their safety as well as efficacy in chemoprevention of SPT among patients with HNSCC. Curcumin, an active ingredient isolated from Curcuma longa, commonly known as turmeric, has demonstrated anti-cancer effect on HNSCC and leukoplakia cell lines through its effect on decreasing proliferation/cell migration and increasing apoptosis through the inhibition of the NF-kB pathway [24]. While a phase I clinical trial by Sharma et al., 2004 and Kanai et al. [25] demonstrated that Curcumin is well tolerated in high doses (8g/day for 3 months), a prospective, randomized clinical trial in a cohort of 223 patients with oral potentially malignant lesions (leukoplakia) conducted by our group [10] showed effective chemoprevention. Metformin, a member of the biguanide family, used for the management of Type 2 diabetes, is reported to have anticancer properties including inhibition of insulin-like growth factor, HER2-mediated and mTOR signaling, inhibition of angiogenesis, and induction of cell cycle arrest/apoptosis [26-31]. An observational cohort study reported that Metformin users are at a lower risk of cancer as compared with Type 2 diabetics on other treatments [15]. This study explores the effectiveness of the combination, the hypothesis being

that Curcumin in combination with Metformin can down-regulate multiple tumorigenic pathways, AKT mTOR, and NF-kB [30, 32, 26, 31, 10] and thereby help reduce the incidence of SPTs of the aerodigestive tract in patients with a previous history of HNSCC (figure 1).

This clinical trial provides extensive opportunities for investigating the possible biological basis of SPT development and the clonality with respect to the primary and/or recurrent tumors. In addition, the exploration of the biological basis might also suggest possible non-invasive candidate biomarkers that can be used to predict susceptibility. The molecular analysis in the trial will include assessment of the clonality of the second primary tumor in comparison to primary/recurrent tumor, primary tumor marker profile, and serum/saliva marker profile to understand susceptibility and occurrence of SPT amongst the four arms. Previously, a study conducted in head and neck cancer demonstrated that the levels of ITPR3, DBI, AHNAK, IGHV3-49, CALML3, ARPC2, DSG3, and KRT37 were significantly associated with a shorter time to second primary malignancy development ($P<0.05$) [33]. Another study reported that TIMP3 ($p=0.007$) and CCNA1 hyper methylation ($p=0.001$) were significantly associated with lower rates of second primary tumor-free survival (log-rank test) [34]. Our study will add to these evidences and help develop a comprehensive prognostic model for SPT/Recurrence in HNSCC by combining clinical, histology, and molecular profiles of the patients.

The study includes a run-in period, which prevents early dropouts due to poor tolerance of Curcumin and/or Metformin. A phase II part is incorporated to test the efficacy and tolerance of combination therapy. Although the sample size is large (n=1500), the study is executed by the head and neck cooperative oncology group (HNCOG), which comprises multiple institutions from different parts of India that will help to achieve the recruitment target. Successful implementation of this study through a cooperative network will enable future collaborative multi-centre HNSCC studies. The secondary endpoints of the trial are also likely to yield valuable basic science information.

In conclusion, chemoprevention with Curcumin and Metformin has the potential to significantly impact the occurrence of SPTs in HNSCC. The recruitment for this multi-centric 2*2 factorial randomized control trial is ongoing with 705 patients recruited in the trial, from the collaborating centers across India. Through the results of our multi-centric phase III randomized control trial, we hope to be able to indicate if these agents are suitable for routine clinical use and better understand the molecular basis of carcinogenesis in this large cohort of patients.

Author Contribution Statement

VK, SN, SS, AS, SI, and MAK contributed to the concept and design of the protocol. SN, VV, AR, and AM contributed to the literature search, writing manuscript, and data analysis. TK, AK, RK, SVN, KT, BMN, RK, SB, RT, SA, JB, SB, SS, NT, and RK helped in the conceptualization of the protocol. TT, SS, and AR contributed to the development of the statistical plan. SS, RK, PC, MAK, and AS contributed to the critical revision and finalizing of the manuscript. All the authors reviewed the finalized manuscript.

Acknowledgements

General

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Approval

The study protocol has been approved by the Narayana Health Medical Research Ethics Committee of the coordinating center; Narayana Health City, Bangalore (Reference number: NHH/MEC-CL-2016-412(A)) on 28 February 2018 and has been registered under CTRI (CTRI/2018/03/012274). The study will follow ethical principles for medical research involving human subjects of the Declaration of Helsinki, adopted by the 18th General Assembly of the World Medical Association which was last revised at the association's 64th General Assembly, in Fortaleza, Brazil in October 2013. All the subjects will be provided informed consent to participate.

Ethical Declaration

Narayana Health Medical Ethical Committee, Narayana Health City, #258/A, Bommasandra Industrial area, Anekal Thaluk, Banglore.

Study Registration

The study is registered in CTRI (CTRI/2018/03/012274)

Conflict of Interest

None of the authors have conflicts of interest to declare.

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