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METHODS AND PROTOCOLS OPEN ACCESS

An Open-Label Phase II Trial of Pembrolizumab, an Immune Checkpoint Inhibitor Alone or in Combination With Oral Azacitidine as Second-Line Therapy for Advanced Head and Neck Squamous Cell Cancers

Vinod K. Ramani[1] | Srimonta Gayen[2] | Radheshyam Naik[3]

[1]Preventive Oncology, Healthcare Global, Bangalore, India | [2]Developmental Biology and Genetics, Indian Institute of Science, Bangalore, India | [3]Medical Oncology, Healthcare Global, Bangalore, India

Correspondence: Vinod K. Ramani (drvinod.r@hcgel.com)

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ABSTRACT

Background and Aims: Sensitivity to immune checkpoint inhibitor (ICI) therapy depends in part on the genetic and epigenetic makeup of cancer cells, and CD8 T-lymphocytes that mediate immune responses. Epigenetics are heritable reversible changes in gene expression that occur without any changes in the nuclear DNA sequence or DNA copy number.

Primary Objective: i. To determine if non-cytotoxic oral azacytidine when combined with pembrolizumab can improve ORRs of ICI treatment in patients with recurrent/metastatic tumors of head and neck region.

Secondary Objectives: i. To evaluate the clinical effectiveness endpoints and toxicity of oral azacytidine when combined with pembrolizumab. ii. To assess the induction of a T-cell response among the study subjects. iii. To examine the hypotheses on the predictive biomarkers of response to pembrolizumab, and the mechanisms of resistance.

Methods: Our trial is a Phase 2 randomized study of immunotherapy drug pembrolizumab given in combination with azacitidine (HMA). The intervention model includes "Parallel assignment," with the primary purpose of the trial being treatment. The primary effectiveness endpoint is overall RECIST-defined response. To accomplish this goal, 232 patients will be randomized 1:1 (116 in each arm), respectively, to azacitidine plus pembrolizumab or pembrolizumab only groups.

Results: In this trial, molecular profiling of tumor and peripheral blood samples will be conducted which will enable in gaining biological insights for survival benefit. The expected primary outcome assessed at a time frame of 2 years includes the objective response rate of patients measured as per RECIST 1.1 criteria. The secondary outcomes assessed at 2 years include progression-free survival, time to progression, overall survival, and incidence of treatment-emergent adverse events.

Conclusion: The findings of this trial will have translational implications, in terms of immune reprogramming induced by epigenetic therapy among a subset of advanced H & N cancer patients in a clinical setting.

1 | Background

Evidence shows that initiating anti-PD-1 monotherapy for patients with recurrent/metastatic head and neck cancers (squamous cell) who have earlier received platinum-based therapies, induces an objective response rate (ORR) which ranges from 13.3% to 18% [1]. Initial responders to immune checkpoint inhibitor (ICI) therapy show secondary resistance subsequently, and lose their response eventually through myriad mechanisms [2]. Over the last three decades, the role of epigenetics in cancer causation is being increasingly researched beyond its genetic basis. Epigenetics are reversible changes in gene expression which are heritable, and they tend to occur without any alteration in the nuclear DNA sequence or DNA copy number [3]. Cellular epigenetic changes such as DNA cytosine methylation and histone modification can result in chromatin remodeling [3]. Research indicates the influence of epigenetic modifiers on the tumor microenvironment (TME) and their influence on promoting an "inflamed phenotype" in the tumors, which can conceivably liaison with immunotherapies [2].

Cytosine–phosphate–guanine (CpG) islands (cytosine bases in DNA) are located in the promoter regions of the gene and are unmethylated in normal cells. The transcribed region (gene bodies) are CpG-poor and are methylated extensively. Tumorigenesis is fostered both by local DNA promoter hypermethylation and non-promoter (intragenic and intergenic regions) hypomethylation [3]. This abnormal pattern includes regional promoter hypermethylation of DNA at the CpG islands (which mutes the expression of TSGs), and global hypomethylation of CpG sites (which promotes chromosomal instability) at repeat elements/retrotransposons. A demethylating agent's ideal focus should be on the methylated promoter regions over heterochromatic DNA repetitive sequences (LINE 1) and the mode of action includes enabling its preferential demethylation [3]. A surrogate marker for overall DNA methylation includes LINE 1 methylation, and its reversal by hypomethylating agents (HMAs) is accompanied by global hypomethylation among both the clinical responders and nonresponders [3]. The sensitization to ICI (PD-1/PD-L1) therapy and reversal of resistance has been evidenced from preclinical data which suggests the epigenetic reprogramming of CD8 T-lymphocytes [2].

In the DNA, the site of methylation is the CpG dinucleotides and the process includes the reversible addition of methyl group to Position 5 of the cytidine ring [3]. Promoter DNA methylation of tumor suppressor genes (TSGs) in cancer cells tends to silence their expression and plays a role in tumor initiation and progression [3]. A correlation exists between DNA cytosine methylation at C5 and gene expression. The aberrant methylation of DNA is targeted by the HMAs for inverting the epigenetic silencing and reactivating the TSGs [4]. The antitumor activity of HMAs is explained by the demethylation of aberrantly methylated CpG-rich gene promoter regions [3]. This results in reactivating the silenced TSGs, and subsequent induction of apoptosis or differentiation, which have the potential to hinder tumor cell viability [3]. As HMAs are cytotoxic at high doses, a low-dose HMA agent tends to reactivate silenced genes and cellular differentiation [3]. During replication, the DNA methyltransferase (DNMT1) maintains the signature DNA methylation pattern on the newly synthesized DNA strands [3]. When this enzyme is blocked, the resultant is a passive demethylation which is dependent on DNA-replication during cell division [3].

This trial will assess the safety of azacitidine and its efficacy when combined with ICI treatment on the natural course of events among a subset of advanced head and neck (H & N) cancer patients in a clinical setting.

2 | Rationale

The genetic and epigenetic makeup of cancer cells impact the sensitivity to ICI therapy, as well as the similar constitution of CD8 T-lymphocytes which mediate immune responses [2]. A novel biomarker in many diseases including H & N cancers includes assessing the methylation of genes involved in their etiopathogenesis. DNMT inhibitors (DNMTis) can entice the T and natural killer (NK) cells to TME through processes such as upregulation of immunostimulatory cytokines, antigen-presentation pathways, and interferon responses, besides contributing to the activation of CD8 T cells [2]. The study will explore the combination of effects, which includes direct effects on malignant cells and indirect facilitation of antitumor immune responses.

The findings of this trial will have translational implications, in terms of immune reprogramming induced by epigenetic therapy among the study subjects.

3 | Hypotheses/Research Question

Our trial is a Phase 2 study of immunotherapy drug pembrolizumab, given in combination with azacitidine. The motive of this study is to assess the antitumor activity of pembrolizumab in combination with azacitidine, for patients with recurrent/metastatic H & N cancer.

For various cancer types, a favorable clinical prognosis after ICI is linked with the abundance and composition of tumor-infiltrating immune cells. The aim of this trial is to restimulate the immune-mediated anticancer effects of ICI in patients with H & N cancer, in a recurrent/metastatic setting.

4 | Study Design

This interventional study is a Phase 2 randomized two arm trial of pembrolizumab alone or in combination with azacitidine, in previously treated patients with advanced cancers of H & N. The primary goal is to compare the efficacy of oral azacitidine as a way of enhancing the antitumor immune response of pembrolizumab when compared with pembrolizumab alone. The primary effectiveness endpoint is overall RECIST-defined response. To accomplish this goal, 232 patients will be randomized 1:1 (116 in each arm) to azacitidine plus pembrolizumab or pembrolizumab only, respectively.

The sample size is calculated based on the assumption that the ORR of immunotherapy (nivolumab) treatment in recurrent/metastatic H & N cancer is 18% [5]. At a 5% α error probability, and a 7% margin of error the estimated sample size is 116 for each arm (232 for both arms).

Type of study: Interventional (Clinical trial)

Study subjects: 232 participants (116 in each arm)

Allocation: Randomized

Intervention model: Parallel assignment

Masking: None (Open Label)

Primary purpose: Treatment

Official title: An Open-label Phase II trial of Pembrolizumab, an ICI alone or in combination with oral azacitidine as second-line therapy for recurrent/metastatic H & N cancers

Study start date: December 2024

Actual primary completion date: June 2027

Actual study completion date: December 2027

The primary endpoint of the trial is ORR. Secondary endpoints include time to progression, progression-free survival (PFS), and overall survival (OS).

Table 1 shows the experimental and control arms of the study, and the interventions provided to each arm. The durg "Azacitidine" is used in the management of myelodysplastic syndrome and acute myeloid leukemia. Evidence [6] indicates the following adverse events (AEs) of this drug which includes cardiac failure, hematologic toxicity, infection, and tumor lysis syndrome. If there is onset of any serious AE before the therapeutic effect becomes apparent, any of the suitable following measures will be adopted (Tables 2–4):

i. Appropriate supportive care.
ii. Dose reduction.
iii. Drug withdrawal.
iv. Discontinued from the trial.

Azacitidine is available in oral and subcutaneous formulations. The regimen approved for oral administration is 300 mg once daily for 14 days in a 28-day cycle, whereas the regimen for subcutaneous dose is 50 mg/m^2/day for 5 days in a 28-day cycle. There exists no evidence for the comparison of the clinical effectiveness of oral and subcutaneous formulations of azacitidine as maintenance therapy for acute myeloid leukemia in remission. The benefits associated with oral formulation include self-administration by the patients at home, as well as the reduced risk for hematological AEs. Subcutaneous formulation needs to be administered by trained hands, and necessitates monitoring for hematological AEs [8].

4.1 | Primary Objective

i. To determine if non-cytotoxic oral azacytidine when combined with pembrolizumab can improve ORRs of ICI treatment.

4.2 | Secondary Objectives

i. To evaluate the clinical effectiveness endpoints (PFS, OS) and toxicity of oral azacytidine when combined with pembrolizumab.
ii. To evaluate the induction of a T-cell response in patients with recurrent/metastatic tumors of H & N cancers on the combination regimen.
iii. To assess the disease control rate (DCR) which is defined as CR, PR, or SD for ≥ 16 weeks.

5 | Outcome Measures

5.1 | Primary

i. ORR (Time frame: 2 years)

The proportion of participants with complete or partial response (PR) to combination treatment with pembrolizumab and azacitidine.

TABLE 1 | Arms and interventions.

Sl. no.	Arm	Intervention/treatment
1	Experimental: Azacitidine is given orally at a fixed dose of 300 mg[a] daily for 14 consecutive days, of every 21-day cycle for 3 cycles, pembrolizumab is given intravenously at a fixed dose of 200 mg (over 1 h) on Day 1 of every 21-day cycle for 12 months or until disease progression	Azacitidine and pembrolizumab
2	Control: Pembrolizumab is given intravenously at a fixed dose of 200 mg (over 1 h) on Day 1 of every 21-day cycle for 12 months or until disease progression	Pembrolizumab only

[a]In case of gastric intolerance, a dose of 100 mg will be given for 21 days.

TABLE 2 | Inclusion and exclusion criteria. The inclusion and exclusion criteria for the recruitment of cases are tabulated in this table. These criteria define the characteristics of the prospective subjects who qualify or disqualify respectively.

Sl. no.	Inclusion criteria	Exclusion criteria
1	Life expectancy of ≥ 12 weeks	Participation in another clinical study with an investigational product during the last 28 days. However, the study allows a concurrent enrollment in an observational clinical study or when in the follow-up period of an interventional study.
2	Histologically or cytologically documented locally advanced (unresectable) or metastatic squamous cell H & N cancer which is incurable	Any previous treatment with a PD1 or PD-L1 inhibitor, including pembrolizumab. The study allows patients who are treated priorly with anti-CTLA4 agents.
3	Have failed prior standard therapy	Prior treatment with Azacitidine, decitabine, or any other hypomethylating agents.
4	Progressed after prior treatment or intolerant of chemotherapy with cancer in recurrent/metastatic setting	History of another primary malignancy.
5	PD-L1 combined positive score (CPS) ≥ 1	Receipt of last dose of anticancer therapy (chemotherapy, immunotherapy, targeted therapy, endocrine therapy, biologic therapy, tumor embolization, monoclonal antibodies, other investigational agent) ≤ 28 days before the first dose of pembrolizumab.
6	Normal/adequate organ and marrow function	Mean QT interval corrected for heart rate (QTc) ≥ 470 ms.
7	Willing and able to comply with the protocol for the duration of study, which includes undergoing treatment, complying with scheduled visits and examination including follow-up	Current or prior use of immunosuppressive medication within 28 days before the first dose of pembrolizumab.
8	Atleast one measurable lesion according to RECIST V1.1.	Previous anticancer therapy related unresolved toxicity CTCAE grade ≥ 2.
9	Atleast one lesion safely accessible for biopsy	Any prior immune-related adverse event (irAE) Grade ≥ 3 while receiving an immunotherapy drug, or any unresolved irAE > Grade 1.
10	Consent for providing tissue from a newly obtained core or excisional biopsy of a tumor lesion	Documented autoimmune disease during the previous 2 years.
11	Consent for providing archival tumor tissue as well as latest biopsy samples, for correlative biomarker studies	Being treated with systemic immunostimulatory agents (viz. interferons, IL 12) within 6 weeks of start of treatment or 5 half-lives of the drug, whichever is shorter.
12	Female subjects of childbearing potential should have 2 negative pregnancy tests, which is verified by the investigator before initiating pembrolizumab or azacytidine	Before initiation of treatment, administration of systemic corticosteroids within the previous 2 weeks. However, iInhaled or low-dose corticosteroids for COPD or asthma is allowed, as are mineralocorticoids.
13	Sexually active females with childbearing potential and having a non-sterilized male partner must consent for practicing true abstinence or the use of atleast two effective methods of contraception for the defined study period	History of allogeneic organ transplant
14	Sexually active males who are non-sterilized and having a female partner of childbearing potential, must consent for true abstinence or the use of atleast two effective methods of contraception for the defined study period	History of hypersensitivity to pembrolizumab or azacitidine, or to any other humanized monoclonal antibody.

(Continues)

TABLE 2 | (Continued)

Sl. no.	Inclusion criteria	Exclusion criteria
15	Life expectancy ≥ 12 weeks	Previous clinical diagnosis of tuberculosis.
16	ECOG performance status < 2	Pregnant female patients or the one who are, breast-feeding, and male or female patients of reproductive potential, who are not using an effective method of contraception.
17	The normal criteria for organ and marrow function of patients are defined below:	Receiving a live attenuated vaccine within 30 days before study inception or within 30 days of initiating the investigational products.
	i. Leukocytes > 3000/mcL	Symptoms of diarrhea due to chronic gastrointestinal conditions during the ensued 3 years before the start of treatment, and/or prior history of gastrectomy or upper bowel resection which interferes with the absorption, distribution, metabolism or excretion of the investigational product. Also, such procedures further the risk of gastrointestinal toxicity for patients.
	ii. Absolute neutrophil count > 1500/mcL	Patients with uncontrolled seizures.
	iii. Platelets > 100,000/mcL	Central nervous system (CNS) diseases that have not been effectively managed such as leptomeningeal disease, metastatic cancers, or cord compression.
	iv. Hemoglobin > 9 g/dL (recent transfusion of blood can meet eligibility criteria as long as post-transfusion value is maintained at ≥ 9 g/dL for 7 days or longer)	Conditions which impede compliance to study requirements or subvert the patient's ability to provide written informed consent such as Uncontrolled infections, symptomatic congestive heart failure, uncontrolled hypertension, unstable angina pectoris, cardiac arrhythmia, active peptic ulcer disease or gastritis, active bleeding diatheses including patients with hepatitis B, hepatitis C or human immunodeficiency virus (HIV), psychiatric illness or impaired social situations.
	v. Total bilirubin ≤ 2.5 × institutional upper limit of normal (ULN)	Any cancer treatment including concurrent chemotherapy, immunotherapy, biologic or hormonal therapy, with the exception of patients with history of noninvasive breast cancer on adjuvant endocrine therapy.
	vi. AST and ALT ≤ 2.5 times × institutional ULN unless liver metastases exist. In this scenario, AST and ALT must be ≤ 5 × IULN	Underwent major surgery within 28 days of the initiation of the study or is still in the recovery stage from prior surgery.
	vii. Creatinine within normal or ≤ 1.5 × IULN or creatinine clearance ≥ 60 mL/min (Cockcroft–Gault formula measured with 24 h urine)	Uncontrolled symptomatic hypercalcemia (> 1.5 mmol/L ionized calcium or calcium> 12 mg/dL or corrected serum calcium > ULN).
	viii. INR or PT ≤ 1.5 × ULN unless the patient is on stable therapeutic dose of warfarin	Patients unable to comply with the procedures of the protocol or willingly provide consent.

Response will be assessed by RECIST 1.1 criteria, where

i. Complete response (CR) = lack of detectable evidence of tumor.
ii. PR ≥ 30% decrease in sum of diameters of target lesions.
iii. Progressive disease (PD) ≥ 20% increase in sum of diameters of target lesions.
iv. Stable disease (SD) ≤ 30% decrease or < 20% increase in sum of diameters of target lesions.

5.2 | Secondary

i. PFS (Time frame: 2 years)

   Number of months from the time of randomization, until radiologic (per RECIST 1.1) or clinical progression or death, whichever comes earlier.

ii. Time to progression (Time frame: 2 years)

   Number of months from the time pembrolizumab is initiated until radiologic (per RECIST 1.1) or clinical

TABLE 3 | GANTT chart of hypomethylation study. GANTT chart as depicted in this table shows the timeline of planned activities in the research project. The benefits include visualizing, scheduling, and tracking the progress of activities. The charts also show the dependency relationships between the activities.

Sl. no.	Activity	Dec '24	Jan '25	Jul '25	Dec '25	Jun '26	Dec '26	Jun '27	Dec '27
1	Development of tools for the study	√							
2	Recruitment of study subjects		√	√	√				
3	Providing the intervention		√	√	√				
4	Collection of biospecimen at the collaborating centers			√	√	√			
5	Analysis at Indian Institute of Science (IISc)[a]			√	√	√	√		
6	Necessary investigations at the hospital (e.g., PET CT scan)			√	√	√	√		
7	Follow-up of patients					√	√	√	
8	Data curation and analysis						√	√	
9	Drafting the manuscript							√	
10	Report submission								√

[a]A duration of 2 months is required for the analysis of each biospecimen at IISc.

progression is noted. Subjects will be censored if they do not progress or die on the date of their last evaluable tumor assessment.

iii. OS (Time frame: 2 years):

Number of months from the time of randomization until death. A subject will be censored when he/she is alive at the last known date. While the subjects are on the study drug, OS will be followed continuously. Estimation will be done by the Kapla–Meier method.

iv. Incidence of treatment-emergent AEs (Time frame: 2 years):

Number of participants who experience adverse events as defined by CTCAE v4.0.

6 | Investigations

In this trial, we will also perform various molecular profiling of tumor and peripheral blood samples, which will enable in gaining biological insights for the survival benefit. These include [2]:

1. Genome-wide DNA methylation profiling (for DNA collected from tumor biopsies).
2. Whole transcriptome analysis.
3. Immune profiling by multiparametric flow cytometry.
4. DNA methylation status of tumor tissue by EPIC methylation array.
5. Methylation status of peripheral blood mononuclear cells by LINE-1 methylation assay.
6. Total RNA sequencing for determining global gene expression changes (for RNA collected from tumor biopsies).
7. Markers of immune activation in circulating tumor cells.
8. Abundance of tumor-infiltrating CD8 T cells within the tumor.

The findings of this trial will have translational implications, in terms of the epigenetic therapy inducing an immune reprogramming among a subset of advanced H & N cancer patients in a clinical setting.

7 | Eligibility Criteria

Age group: 18 years and older

Sex: Both males and females

Healthy volunteers: Not accepted

Written informed consent: Taken

Eastern cooperative oncology group (ECOG) performance status < 2

The various laboratory assessments done on tumor and blood samples are shown in Figure 1. These tests include assessment for methylation status of both DNA and RNA, as well as immune activation [9, 10].

8 | Institutional Support

Health Care Global (HCG) has a network of 22 comprehensive cancer centers in India and abroad. It is the largest private cancer care provider in India. The aim is to provide state-of-the-art cancer care. In this regard, each center is equipped with capital resources and supplemented with a business system and

TABLE 4 | Cost of the study. The cost for each component of the study is enlisted in this table. This includes the expenditure for supplementing the drug azacitidine, the relevant laboratory investigations at baseline and endline of the study.

Sl. no.	Item	Availability	Duration	Cost per unit (INR)	Total cost (INR)
1	Drug (azacitidine)	14 tablets/strip	3 months (1 strip/month)	22,000/strip	$14 \times 3 \times 22{,}000 = 9{,}24{,}000$
2	Investigations				
	Baseline [7]: i. Genome-wide DNA methylation profiling (for DNA collected from tumor biopsies). ii. Whole transcriptome analysis. iii. Total RNA sequencing for determining global gene expression changes (for RNA collected from tumor biopsies). iv. Markers of immune activation in circulating tumor cells. v. Abundance of tumor-infiltrating CD8 T cells.		2 months/sample	Rs. 90,000/sample	$232 \times 90{,}000 = 2{,}08{,}80{,}000$
	Endline [7]: (1 month after completion of 3-month course of azacytidine treatment) vi. Immune profiling by multiparametric flow cytometry, on peripheral blood monomuclear cells (PBMC) vii. DNA methylation status of tumor tissue by EPIC methylation array, viii. DNA Methylation status of PBMC by LINE-1 methylation assay by pyrosequencing (for genome-wide demethylation), ix. Markers of immune activation in circulating tumor cells, x. Abundance of tumor-infiltrating CD8 T cells				
3	Cost of the project				2,18,00,000

Note: Conversion rate: 1 United States Dollar (USD) = 84 Indian rupees (INR).

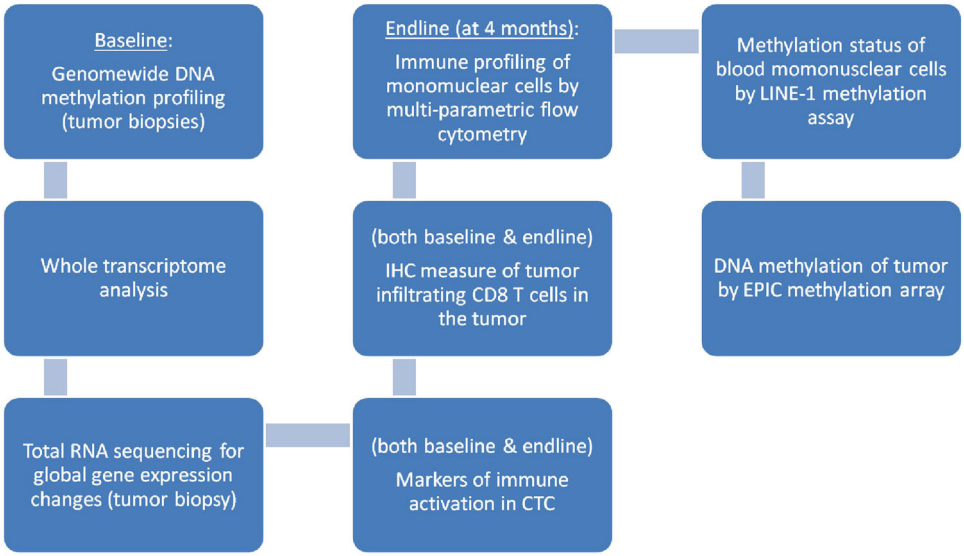

FIGURE 1 | Molecular profiling of tumor and blood samples.

management expertise for providing patient-focused health services. An integrated approach to cancer care has been facilitated by the hub and spoke model health system. Annually, HCG centers cater to the needs of > 120,000 cancer patients and the study subjects will be recruited from these centers.

Indian Institute of Science will conduct the tests on the biological samples. The diverse areas of research conducted at the Department of Developmental Biology and Genetics include bacterial and human genetics, developmental biology, mammalian reproduction, signal transduction, cancer, and stem cell biology. The department is well-equipped to conduct molecular-level studies of cellular functioning in normal and pathological conditions.

i. Genome-wide DNA profiling:

   Components: Illumina Human Methylation EPIC (EPIC) array Including DNA isolation kit, EZ DNA Methylation Kit

   IISc will profile genome-wide DNA methylation (CpG) in tumor biopsies using the Illumina Human Methylation EPIC array (Illumina, CA). In brief, we will isolate the DNA from the tumor using Qiagen DNA purification kit and will quantify the DNA using Qbit fluorometers (Invitrogen). Next, we will use 250–500 ng DNA for bisulphite treatment using EZ DNA Methylation Kit (Zymo), following the Illumina manufacturer's protocol. Next, we will use this bisulphite converted DNA to profile the methylation using Methylation EPIC array chip. We will process the data using tool available in R package.

ii. Whole trancriptomic profiling:

   Components: RNA isolation kit, bioanalyzer charges, NEB Next ultra-library preparation kit, charges for Illumina Novaseq 6000

   For transcriptomic analysis, we will isolate the total RNA using Trizol method from tumor biopsies. RNA quality will be checked using Agilent bioanalyzer. RNA samples having RIN value greater than 8.0 will be used for library preparation using NEB Next ultra-library preparation kit following the manufacturer protocol. Library will be sequenced through Illumina Novaseq. 6000 platform. RNA-seq data will be analyzed using DE-seq 2 package.

9 | Coordination Between Clinical, Laboratory and Data Management Procedures

The electronic data capture tool REDCap (Research Electronic Data Capture) will be used to collect and manage the study data. The interface provided by this web-based application will enable the entry of validated data, tracking the manipulation of data, automated export process to common statistical packages, and avenues for importing data from external sources.

10 | Future Plan

Depending on the outcomes of this Phase II study, we intend to conduct a Phase III trial on a multi-institutional cohort pan-India. A positive result in the Phase III trial will enable a practice changing outcome, with a presumed benefit of 10%–15% improvement for the combination (immunotherapy + azacitidine) regimen when compared to the solo immunotherapy regimen in the setting of recurrent/metastatic head and neck cancers.

Author Contributions

Vinod K Ramani: methodology, writing–original draft, investigation, funding acquisition, formal analysis, software, resources, data curation. **Srimonta Gayen:** project administration, investigation, writing–review and editing, methodology, resources, data curation. **Radheshyam Naik:** supervision, conceptualization, investigation, funding acquisition, writing–review and editing, validation, project administration.

Acknowledgments

This clinical trial has been submitted for registration with the "Clinical Trials Registry of India." We have adhered to the relevant EQUATOR guidelines, and the reporting method is referenced in the abstract and methods section of the paper. Manohar Mhaske: statistician, technology, healthcare, big data analytics, Gurugram, India.

Ethics Statement

This trial has been approval by the Institutional Research Ethics Committee, Healthcare Global, Date: 19 April 2024, Id: EC/713/24/8.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Given the privacy concerns, data can be accessed by writing to the corresponding author.

Transparency Statement

The lead author Vinod K. Ramani affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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