Alisertib and Pembrolizumab in Rb-deficient Head and Neck Squamous Cell Carcinomas

Faye M. Johnson[1,3], Madison P. O'Hara[1,3], Alexandre Reuben[1], J. Jack Lee[2,3], Yun QCng[2], Hai T. Tran[1], Soma Ghosh[1], Lacin Yapindi[1], Peixin Jiang[1], George Blumenschein[1], Jagannadha Sastry[1,3]

[1]Department of Thoracic/Head & Neck Medical Oncology and [2]Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, Texas; [3]The University of Texas Graduate School of Biomedical Sciences, Houston, Texas.

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background and Rationale

- Head and neck squamous cell carcinoma (HNSCC) is a common and lethal cancer for which better therapy is needed (1).
- Although immune checkpoint therapy (ICT) has a striking effect in some HNSCC patients, the majority have intrinsic or acquired resistance.
- Human papilloma virus (HPV) is a common cause of HNSCC that leads to degradation of the Retinoblastoma (Rb, *RB1*) protein.
- Rb-deficient cancers are hyper dependent upon Aurora kinases for survival (2-6).
- Alisertib (MLN8237, TAK8237) is a selective small molecule inhibitor of Aurora A kinase.
- Alisertib leads to immunogenic cell death (ICD) in HPV+ cancer cells (6)
- This trial tests the **hypothesis** that Aurora A inhibition will lead to apoptosis and ICD in HPV+ HNSCC leading to host T cell engagement and increased sensitivity to ICT.

Study Objectives

Primary objectives:

- Phase I: To determine the recommended phase II dose of the combination of alisertib and pembrolizumab.
- Phase II: To determine the overall response rate and progression free survival (PFS) of patients with recurrent or metastatic Rb-deficient HNSCC treated with the combination.

Secondary objectives:

- To evaluate safety of the combination.
- To determine the overall survival (OS) in treated patients.
- To determine the relationship between response and pharmacokinetics (PK), biomarkers, and the effect of the treatment on HPV-reactive T cells.

Statistical Design

Phase I: Bayesian optimal interval design with a target toxicity rate for the maximum tolerated dose (MTD) of 0.3. Patients enrolled in cohorts of size 2. If the observed dose-limiting toxicity (DLT) rate at the current dose is ≤ 0.236, the next cohort of patients will be treated at the next higher dose level; if it is ≥ 0.359, the next cohort of patients will be treated at the next lower dose level; otherwise, the next cohort of patients will be treated at the same dose.

Phase II: Bayesian Optimal Phase 2 (BOP2) design. The assumptions are that the null response rate is 5% and the target response rate is 20%. Fourteen patients were enrolled initially. Per design, because there were no responses, the trial was terminated due to lack of efficacy.

Study Design and Eligibility

- Phase I Eligibility
  - Solid tumor without standard life-prolonging therapy.
  - No requirement for Rb deficiency.
- Phase II Eligibility
  - Rb-deficient HNSCC (HPV+ or Rb immunohistochemistry negative)
  - Progressed on prior anti-PD1 without severe immune-related adverse events
- Design
  - Pembrolizumab 200 mg IV q 3 weeks
  - Alisertib 30-50 mg po BID x 7 days every 21 days
  - Imaging baseline and every 2 cycles
  - PK in first 12 patients on phase II
  - Blood for HPV cfDNA, cytokines, E6/E7 tetramers, flow cytometry in phase II.

Dose Level	Alisertib Dose
-1	30 mg po BID x 7 days every 21 days
1	40 mg po BID x 7 days every 21 days
2	50 mg po BID x 7 days every 21 days

Phase I – patient characteristics

Subject Number	Cancer	Prior IO	Dose Level	Race	Sex	Age	Response	PFS (Days)	PDL1 CPS Score	TP53	Next Generation Sequencing
1	SCLC	Y	1	White	Female	35	SD	95	40	wt	CCND1 amplification, SPEN, TSC2
2	SCLC	Y	1	Black	Female	61	SD	245	ND	ND	ND
3	HPV+ OPC	Y	2	White	Male	56	SD	209	3	wt	AKT3, CREBBP, FAT1, GEN1, HDAC2, MST1R, NOTCH3, NRAS, PLCG2, TCF3, XPO1
4	SCLC	N	2	White	Female	68	PD	45	0	ND	ND
5	SCLC	Y	1	White	Male	67	PD	46	ND	ND	ND
6	HPV- OPC	Y	1	White	Male	61	SD	84	5	Mutant	TP53, KEAP1, MET, MSH6
7	CASTLE	Y	1	Black	Male	52	SD	811	7	wt	KDM6A
8	Salivary[1]	N	1	White/Hispanic	Male	40	SD	290	2	wt	PIK3CA
9	Acinic Cell	Y	2	White	Female	55	PD[2]	28	ND	Mutant	TP53, GNA11, RET, SMO, CCND1, MAPK3, ESR1
11	HPV-HNSCC	Y	2	White	Male	62	SD[3]	64	50	Mutant	CDKN2A, NOTCH3, RHOA, SETD2, TP53, TERT

[1]Myoethelial carcinoma
[2]Off study for declining performance status designated PD
[3]Off study for patient preference designated date of PD
CASTLE, thyroid carcinoma showing thymic-like differentiation
HNSCC, head and neck squamous cell carcinoma
OPC, oropharynx cancer
SCLC, small cell lung cancer
HPV, human pappillloma virus
SD, stable disease
PD, progressive disease
IO, anti-PD1 or anti PDL1 immunotherapy
ND, not done

Phase II – patient characteristics

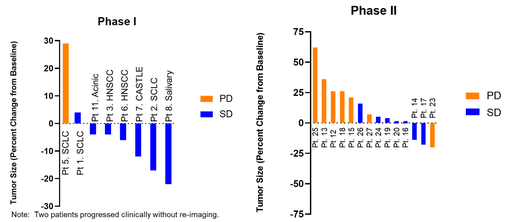
Subject Number	Race	Sex	Age	Response	PFS (Days)	PDL1 CPS	HPV type	TP53	Next Generation Sequencing	TMB	MSI
12	White	Male	62	PD	44	15	NR	Y236S	FANCA, NF1, TERT, TSC2	ND	ND
13	White	Male	75	PD	42	5	16	wt	BRAF G596R	ND	ND
14	White	Male	51	SD	373	ND	16	wt	RAF1 D19V; Rb1loss, PTEN p.N323fs*2	3.3	ND
15	White	Male	74	SD	41	>20	16	wt	BRCA1, CENPA, CYLD, EGFR, ERCC3, IGFR, PBRM1, RB1	3	Stable
16	Asian	Male	53	SD	252	<1	16	wt	ARID1, FGFR2, HRAS, TPMT	ND	ND
17	White	Male	61	SD	384	30	16	wt	B2M, CBFB, IRF4, LIG4, NCOR1, PIK3CB	5	Stable
18	White	Male	69	PD	40	5	16	wt	PIK3CA, RHOA, STK11	ND	ND
19	White	Male	59	SD	66	15	16		Not tested	ND	ND
20	White	Male	80	SD	83	2	16	wt	CYD, EPHA3, FAT1, RBM10	2	Stable
23	White	Male	63	PD	42	20	16	wt	None identified	low	Stable
24	White	Male	67	SD	125	5	16	wt	FBXW7, HERC2, POLA1, TRAF2	3	Stable
25	White	Male	70	PD	41	70	16	wt	None	ND	ND
26	White	Male	67	PD	41	1	16	wt	NOTCH1	0	Stable
27	White	Male	51	PD	42	100	33	wt	None	ND	ND

NR, no result from cfDNA
MSI, Microsatellite Instability
TMB, tumor mutation burden (Mutations per megabase)

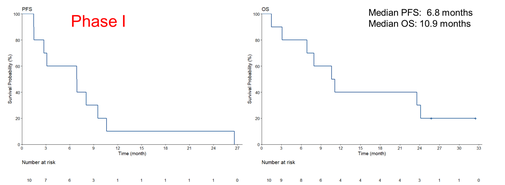
Phase II Results - Overview

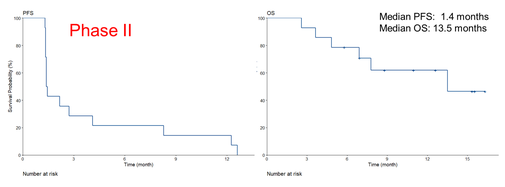
- All HPV+ HNSCC and prior pembrolizumab
- All started at dose level 1
- Two patients dose reduced alisertib at cycles 13 and 16 for cytopenias.
- Pembrolizumab discontinued in patient 17 at cycle 12 due to elevated liver function tests that subsequently normalized.

Response and Survival



Note: Two patients progressed clinically without re-imaging.

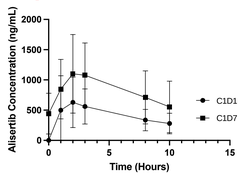




Adverse Events – Total number of events regardless of attribution (n>1 or grade >2)

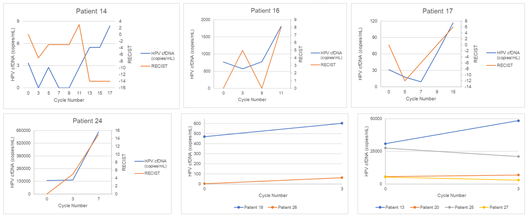
	Grade 2	3	4
ALT increased	2	1	
Alopecia	3		
AST increased	1	1	
Bilirubin increased	2		
Diarrhea	2	1	
Dry skin		1	
Dyspnea	2	1	
Fatigue	7		
Hypertension		1	
Hypokalemia		2	
Hypotension		1	
Hypoxia		1	
Lung infection			1
Mucositis oral	3	1	
Nausea	1	2	
Pain	2		
Pruritus	2		
Rash acneiform	1	1	
Respiratory failure			1
Sepsis			1
Skin peeling		1	
Skin hyperpigmentation		1	
Tracheitis			1
Anemia	23	4	1
Febrile neutropenia		2	
Lymphocyte count decreased	19	13	4
Neutrophil count decreased	5	6	7
Platelet count decreased	5	2	1
White blood cell decreased	9	8	6

Alisertib pharmacokinetic (PK) parameters are similar to prior published studies



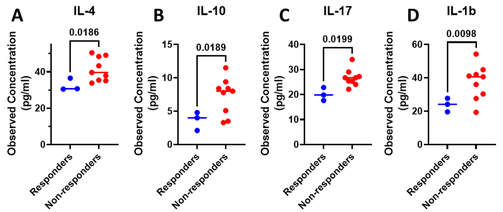
PK parameters were assessed on patients that were dosed at 40 mg orally every 12 hours. Mean PK population plot of alisertib plasma concentrations over dosing period (bars, standard deviation) on Cycle 1: days 1 and 7 (left panel). An increased in alisertib exposure over the first week reaching steady state with an accumulation index of 1.54 on Day 7.

Levels HPV cell free DNA (cfDNA) increased when patients progressed



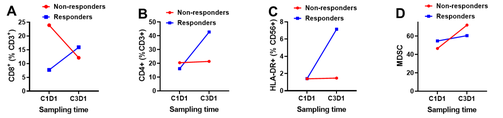
cfDNA HPV was measured using droplet digital PCR (ddPCR) longitudinally in ten patients. Values ≥ 5 are considered positive and ≥ 16 copies/mL are considered quantifiable. Tumor size was measured using Response Evaluation Criteria in Solid Tumors (RECIST) and graphed as percent change from baseline for those four patients who remained on therapy for over 4 months.

Baseline plasma TH2 cytokine response predicts outcome



Plasma samples collected at the baseline from the participants of the trial were subjected to Luminex analyses to profile various cytokines and chemokines. "Responders" were defined as those patients with a PFS > 6 months. Shown are the IL-4 (A) and IL-10 (B), the two TH2 cytokines along with the TH-17 cytokine IL-17 (C) and the inflammatory cytokine IL-1b (D) at significantly higher levels in non-responders compared to responders (significance calculated using unpaired t test with Welch's correction).

Changes in the circulating levels of immune cells correlate with outcome



Blood samples collected at the baseline (C1D1) and after two rounds of treatment (C3D1) from the participants of the trial were subjected to polychromatic flow cytometry analyses to profile various immune cells subsets. "Responders" were defined as those patients with a PFS > 6 months. Shown are the median values of CD8 (A) and CD4 (B) T cells, highly activated subset of NK cells (C), and myeloid derived suppressor cells (MDSC) (D) differentiating non-responders from responders

Biomarkers of Response – Phase II

- No prospective tumor biomarkers correlated with PFS or OS
  - PDL1 CPS score (p = 0.59 and 0.96)
  - Mutations were heterogeneous precluding formal analysis.
  - TMB and MSI were not routinely collected
- Drug exposure did not correlate with PFS: Cmax – p = 0.67 and AUC – p = 0.58

Summary

- Alisertib (40 mg) combined with pembrolizumab was well-tolerated in patients with HNSCC.
- Toxicity was predominantly hematologic as expected.
- Dose reductions only required with prolonged treatment.
- One patient with CASTLE remained on therapy for over 2 years.
- Three HPV+ HNSCC patients remained on therapy for over 8 months.
- HPV cfDNA levels increased at the time of PD.
- PDL1 did not predict PFS.
- PK results suggest that pembrolizumab does not affect alisertib PK.
- Baseline cytokines and changes in circulating immune cells correlated with response.

Conclusions and Future Directions

- This combination was well tolerated with expected toxicity.
- Several patients who had previously progressed on immunotherapy had prolonged SD, supporting our hypothesis that Aurora A inhibition can reverse immunotherapy resistance in Rb-deficient HNSCC.
- However, overall clinical activity was modest, and the trial closed for futility.
- Studies with HPV-reactive T cells are on going.
- Future research will focus on mechanisms to increase ICD and apoptosis in Rb-deficient cancer cells treated with alisertib (6).

References

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