Abstract 6065: Multimodal detection in plasma of molecular residual disease (MRD) in locally advanced head and neck squamous cell carcinoma (LA-HNSCC)

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BACKGROUND

- Up to 30% of the patients (pts) with LA-HNSCC can relapse despite intensive therapy[1].
- MRD detection during follow up (FU) may predict relapse, e.g. human papilloma virus (HPV) DNA in p16+ oropharyngeal cancer (OPC) pts or circulating tumor DNA (ctDNA) in all LA-HNSCC pts post definitive therapy: radiation (RT), chemoradiation (CRT) or surgery (Sx)[2,3].
- MRD detection using multiple assays after definitive RT/CRT has not been reported; and limited MRD data exist post Sx.

METHODS

- Pts with high risk LA-HNSCC treated with curative intent were included and plasma samples were collected at 2 follow up timepoints in the PRE-MERIDIAN study (NCT04599309) (Figure 1). All pts have radiological and clinical assessment at follow up 2 (FU2) with no evidence of disease. Serial sampling past FU1 and FU2 was not part of the intention of this study.

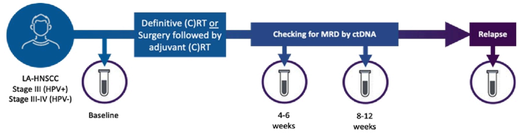

Figure 1. PRE-MERIDIAN study design

- Tumor informed ctDNA analysis was performed using a personalized amplicon based assay (RaDaR®) that targets patient specific somatic variants identified by whole exome sequencing of matched tissue. ctDNA is reported as estimated variant allele frequency (eVAF).
- Tumor naïve ctDNA was analysed using CAncer Personalized Profiling by deep sequencing (CAPP-seq). A panel covering HNSCC variants and stringent filtering (Mutect2, PASS and GnomAD allele frequency < 0.1%) was used to calculate VAF.
- HPV DNA detection was performed using HPV-sequencing (HPV-seq) in all pts while digital PCR (dPCR) was performed only in p16+ OPC pts.
- Relapse free survival (RFS) was considered from the start of definitive treatment. Assays were compared using Spearman correlation.

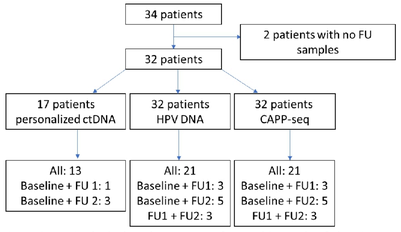

Figure 2. Patients included and assays performed in each of the timepoints

RESULTS

Characteristics	Patients (%)
Gender	
Male	25 (78)
Female	7 (22)
Stage (AJCC 8th edition)	
III HPV+	16 (50)
III HPV-	7 (22)
IV A-B	8 (25)
IV C	1 (3)
Primary Site	
Oropharynx (OPC)	23 (72)
Larynx	5 (16)
Oral Cavity (OC)	3 (9)
Hypopharynx	1(3)
Papilloma Virus (p16+)	
Positive	17 (53)
Negative/Not done	15 (47)
Treatment	
Surgery	1 (3)
Surgery + RT	2 (6)
Surgery + CRT	1 (3)
Definitive RT	3 (9)
Definitive CRT	25 (79)
Cisplatin	
Weekly	12 (46)
High Dose	14 (54)
Non Applicable	6

Table 1. Main characteristics and treatment of the evaluable population in PRE-MERIDIAN (N=32).

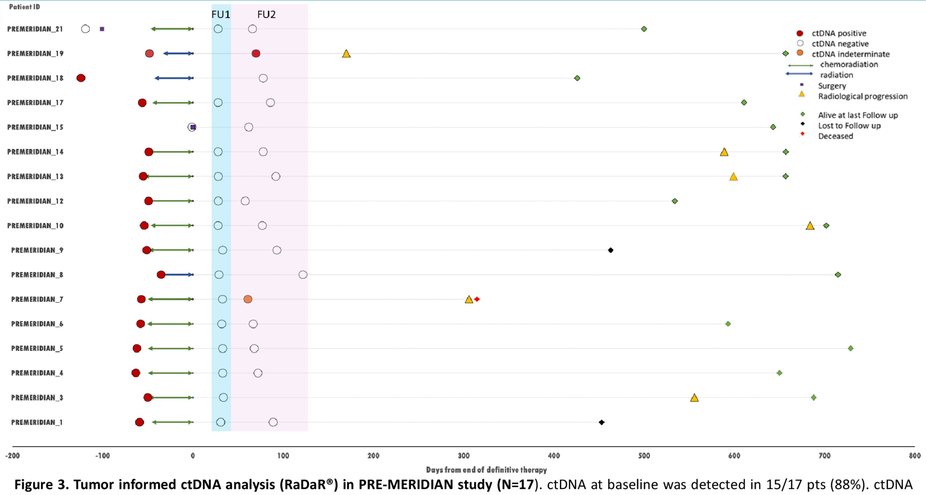

Figure 3. Tumor informed ctDNA analysis (RaDaR®) in PRE-MERIDIAN study (N=17). ctDNA at baseline was detected in 15/17 pts (88%). ctDNA was detected in one patient (PREMERIDIAN_19) in FU2 sample (eVAF=0.004%) with a significant lead time to radiological progression (100 days). In a second patient (PREMERIDIAN_7) FU2 sample was indeterminate (eVAF=0.001%) and a significant lead time to radiological progression (245 days). A third patient (PREMERIDIAN_3) was considered negative at the only available sample (FU1) but eVAF was the highest among negative (0.0001%), recurred 494 days later. Additional 3 patients recurred during the second year of follow up but ctDNA was not detected at FU1 or FU2.

Patient	19	22	7	3	14	13	10
Primary	Larynx	OC	OPC	OPC	OPC	Larynx	OPC
Stage	III	III	III	III	III	III	III
p16	Negative	Negative	Positive	Positive	Positive	Negative	Positive
Treatment	RT	Sx + RT	CRT	CRT	CRT	CRT	CRT
RFS (days)	203	228	356	605	638	653	731
Site of recurrence	Local	Local	Distant (lung)	Distant (lung)	Local + distant (lung)	Local	Local
Alive	Yes	No	No	Yes	Yes	Yes	Yes
RaDaR	**FU2**	NA	FU1/**FU2**	FU1	FU1/FU2	FU1/FU2	FU1/FU2
CAPP-seq	FU2	FU1/FU2	FU1/FU2	**FU1**	**FU1/FU2**	FU1/FU2	FU1/FU2
HPV DNA	*FU2*	*FU1/FU2*	**FU1/FU2**	**FU1**	FU1/**FU2**	*FU1/FU2*	FU1/**FU2**

Table 2. Characteristics of pts with recurrence in the PRE-MERIDIAN study. Seven pts have recurred with a median follow up duration of 18.3 months (5.1-25.9). Clinical characteristics of these pts are displayed. Samples available for each analysis (RaDaR®, CAPP-seq and HPV DNA) are also summarized, in **bold** those results positive. In *italics*, expected negative results for p16 negative HNSCC. NA: Not available.

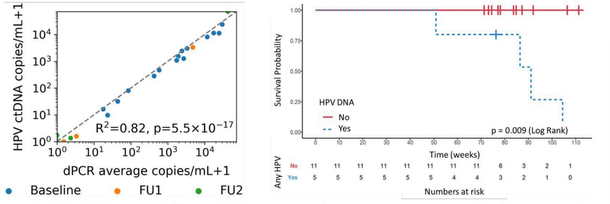

Figure 4. HPV DNA using dPCR and HPV-seq. All p16+ pts have detectable HPV DNA at baseline by both methods (n = 15 pts). There is a high correlation between both assays (left panel, N=44), especially at baseline (r=0.98), and slightly lower at FU2 (r=0.66). HPV DNA detection at FU2 using HPV-seq predicted for a poorer RFS (right).

	FU1 HPV-seq	FU1 dPCR	FU2 HPV-seq	FU2 dPCR	Recurrence
PREMERIDIAN_3	**0.92**	**2.35**	-	-	**YES**
PREMERIDIAN_7	**0.24**	0	**1.20**	0	**YES**
PREMERIDIAN_10	0	0	**0.18**	0	**YES**
PREMERIDIAN_14	0	**0.60**	**0.51**	**1.34**	**YES**
PREMERIDIAN_29	**0.25**	0	0	0	NO

Table 3. Pts with any detectable HPV DNA (copies/ml) at FU1 and/or FU2 using HPV-seq and dPCR. All four pts with clinical recurrence showed HPV DNA detection in FU1 or FU2 using HPV-seq while only two pts with clinical recurrence showed HPV DNA using dPCR.

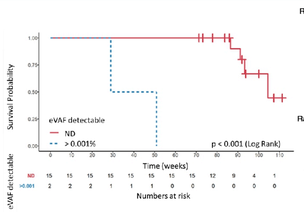

Figure 5. Bespoke ctDNA detection and RFS. eVAF >0.001% at FU can predict relapse in the first year (n = 17 pts).

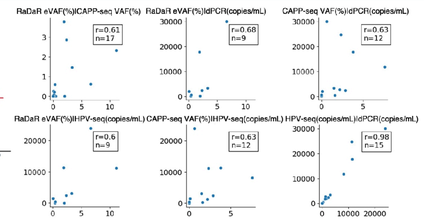

Figure 6. Baseline correlation between methods. High correlation is observed between RaDaR® assay and CAPP-seq at baseline but not seen at FU1 or FU2 (data not shown).

	Method	N	N Relapse	N Positive	N True positive	PPV (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
FU1	RaDaR®	14	5	0	0	-	0	100	64.3
	CAPP-seq	24	5	4	2	50	40	89.47	79.17
	HPV-seq	14	4	3	2	66.67	50	90	78.57
	dPCR	14	4	2	2	100	50	100	85.71
FU2	RaDaR®	16	5	2	2	100	40	100	81.25
	CAPP-seq	26	5	3	1	33.33	20	90.48	76.92
	HPV-seq	13	3	3	3	100	100	100	100
	dPCR	13	3	1	1	100	33.33	100	84.62
FU1/2	RaDaR®	17	6	2	2	100	33.33	100	76.47
	CAPP-seq	29	6	4	2	50	33.33	91.3	79.31
	HPV-seq	15	4	5	4	80	100	90.91	93.33
	dPCR	15	4	2	2	100	50	100	86.67

Table 4. Detection of MRD at FU1 and FU2 using different approaches. These numbers are small and some pts are only evaluable for one test. The pt with indeterminate ctDNA using RaDaR® assay is considered positive in this table. PPV: positive predictive value.

CONCLUSIONS

- HPV DNA and ctDNA can be detected in LA-HNSCC before and after definitive therapy.
- The RaDaR® assay may detect MRD in pts who relapse within 1 year after RT/CRT with a significant lead time while CAPP-seq may not.
- HPV-seq may be more sensitive than dPCR to detect HPV DNA in MRD.
- Validation in an interception study is planned (MERIDIAN; NCT04599309).

References

1. Cooper et al. Postoperative Concurrent Radiotherapy and Chemotherapy for High-Risk Squamous-Cell Carcinoma of the Head and Neck. NEJM 2004;350(19):1937-44.
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Acknowledgments

We would like to thank the patients and their families for their participation and contribution. This study was performed under the auspices of the LIBERATE study (NCT03702309), an institutional liquid biopsy program at the University Health Network supported by the BMO Financial Group Chair in Precision Cancer Genomics (Chair: Dr Lillian Siu). Additional support for the project was made possible by NeoGenomics.

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