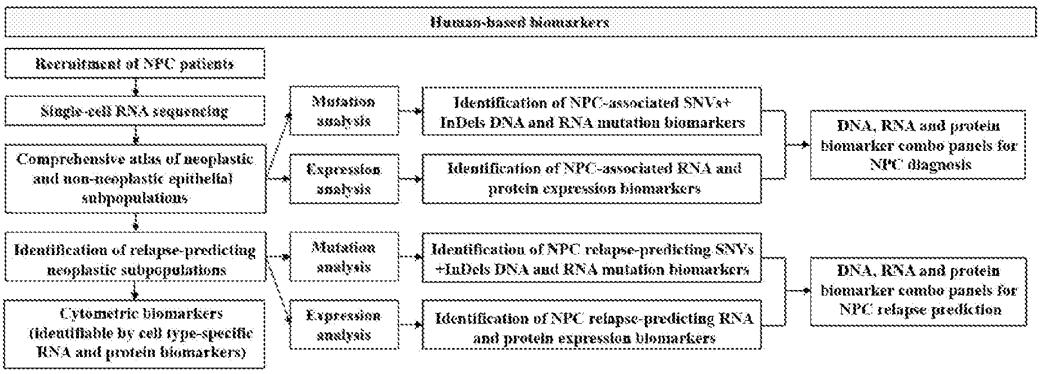
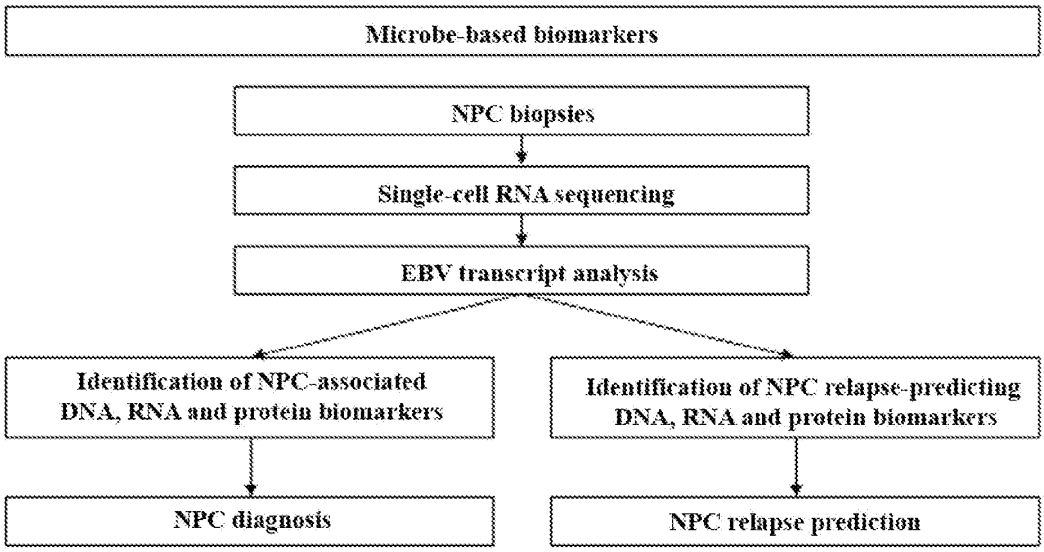
[Fig. 1A]

Rule 26, 10.01.2025

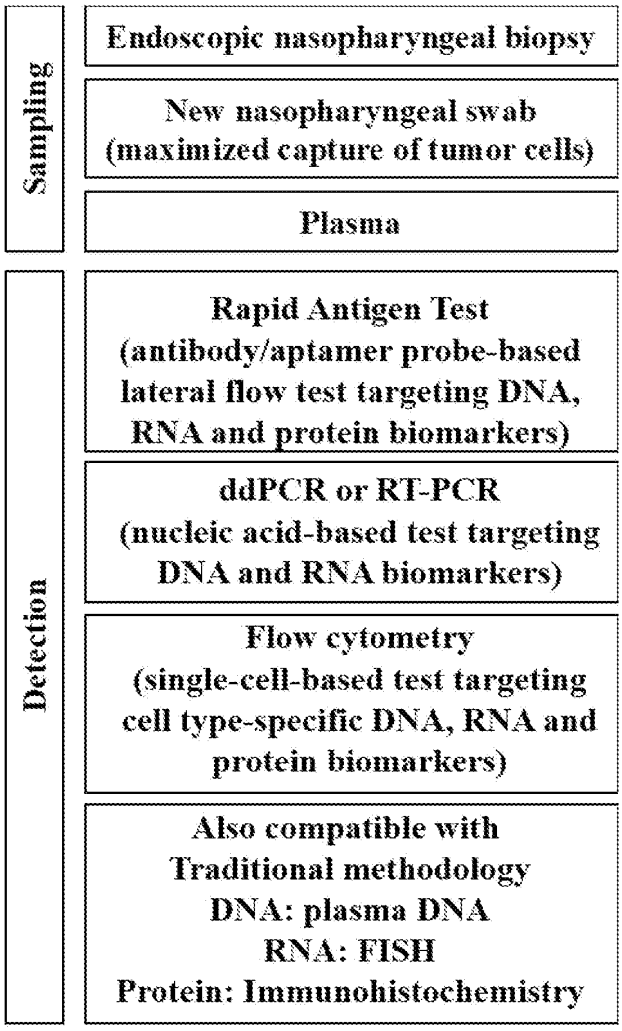


[Fig. 1B]

Rule 26, 10.01.2025

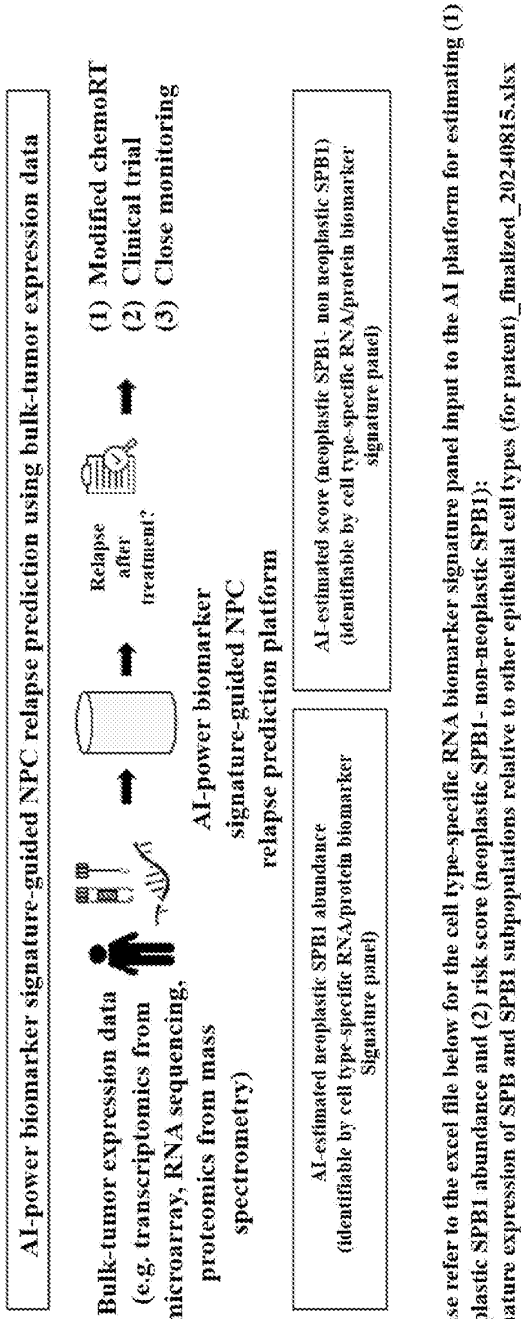


[Fig. 1C]



Rule 26, 10.01.2025

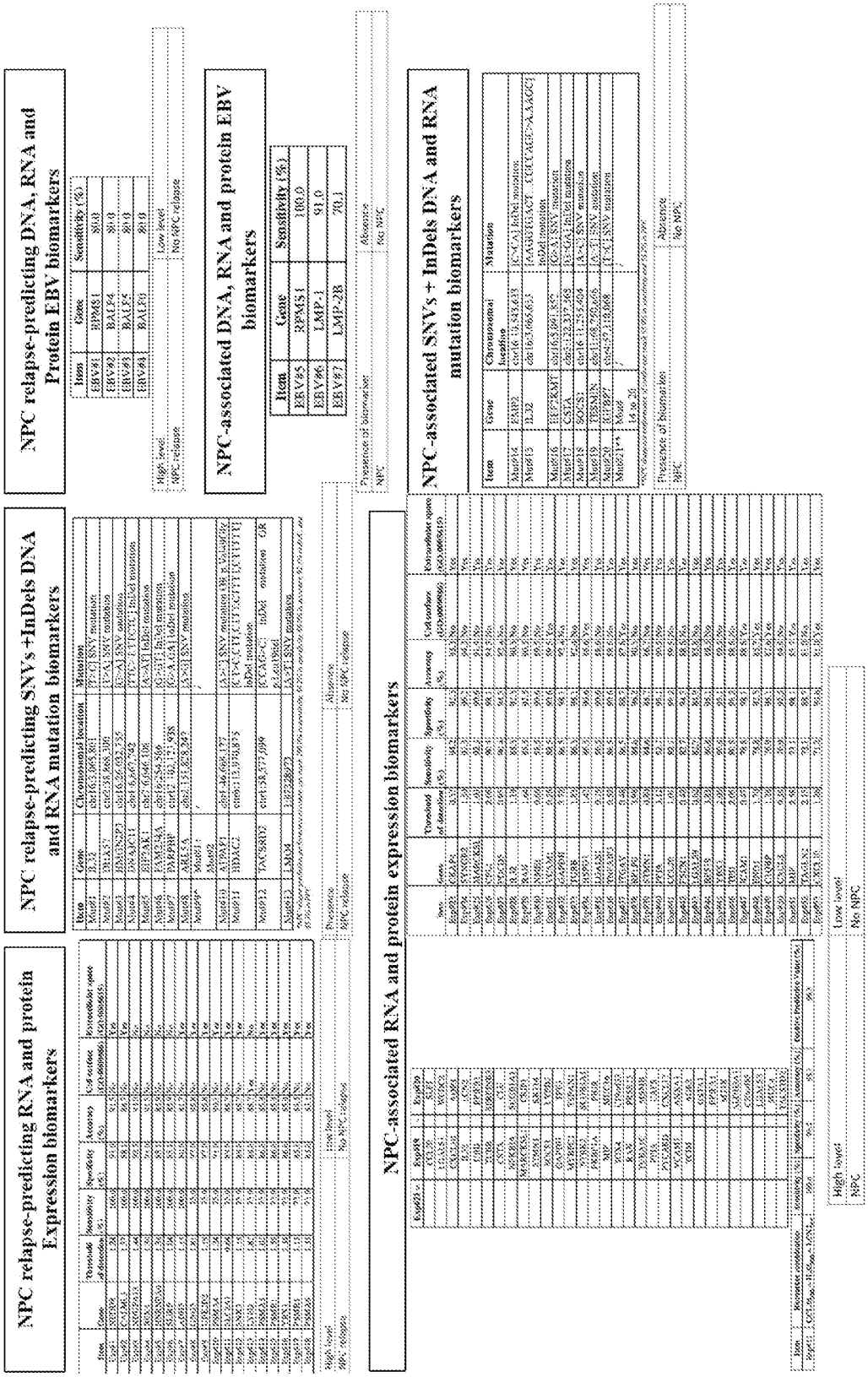
[Fig. 1D]



Please refer to the excel file below for the cell type-specific RNA biomarker signature panel input to the AI platform for estimating (1) neoplastic SPB1 abundance and (2) risk score (neoplastic SPB1- non-neoplastic SPB1):
Signature expression of SPB and SPB1 subpopulations relative to other epithelial cell types (for patent)_finalized_20240815.xlsx

Rule 26, 10.01.2025

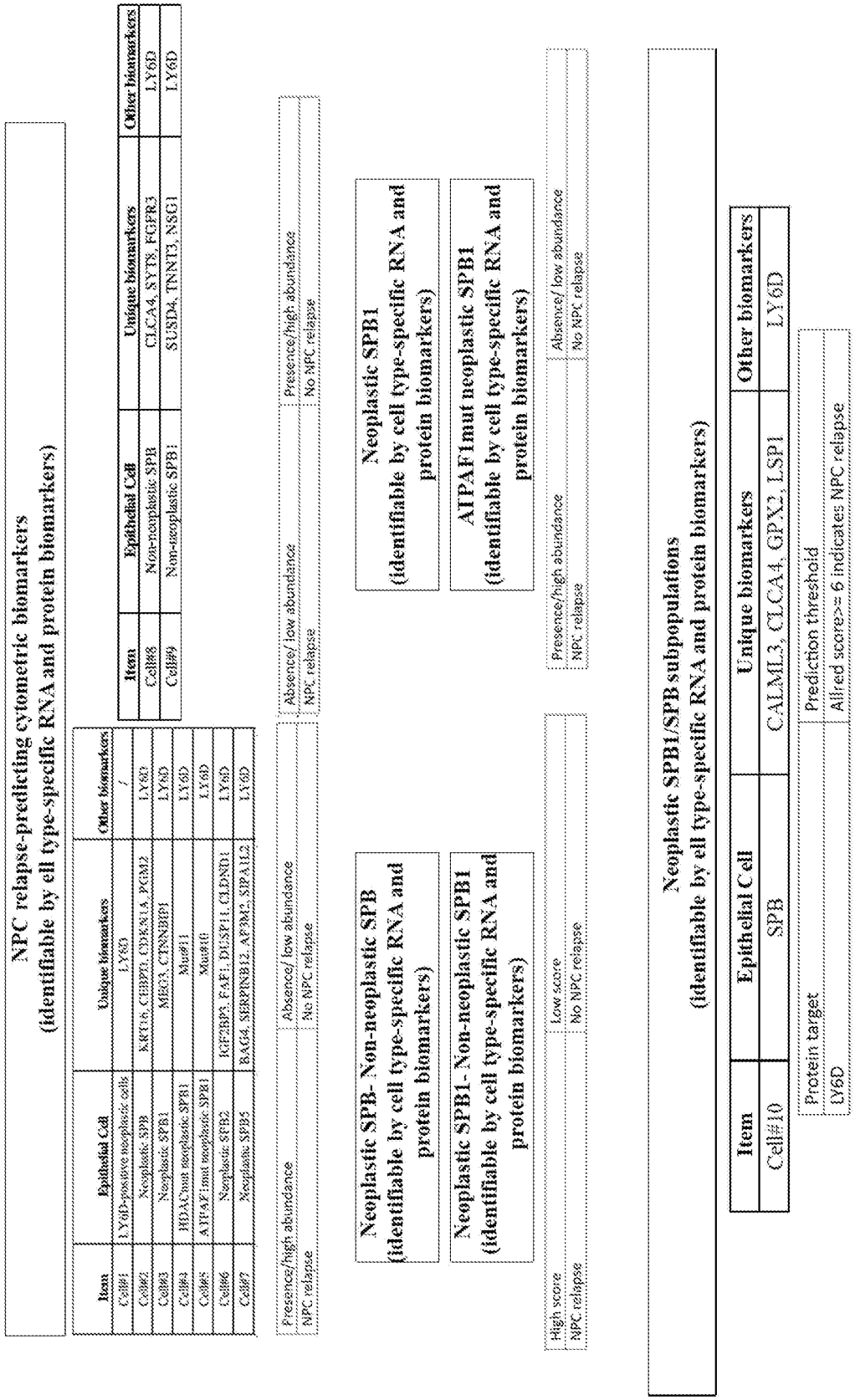
[Fig. 1E]



Rule 26, 10.01.2025

[Fig. 1F]

Rule 26, 10.01.2025



Rule 26, 10.01.2025

[Fig. 1G]

Prognosis	Change	Protein Biomarkers (additional from mass spectrometry analysis)
Relapse	Higher	DHTKD1, PRMT9, SEH1L, MROH1, MED15, NCAN, GLS, DENND5B, COX8A, AUP1, GAN
Relapse	Lower	HMGB2, SNRPF, SRP14, RANBP2L, SAMHD1, BART3, IFI16, CROCC, NCF1B, SLIT1, SRSRGF, IGHV3-53, ANTXR1, SPR42, SLC9A1
De Novo Metastasis	Higher	PRM2, CDH15, PPEPL1, DUSP19, BPNT1, LRRC75A, USP47, MAGT1, TARDBP, NDUFA12, ATOX1, MOSPD2, HBM, VTI1A, NFE2, MTRF5, IGFBP1, OGG, PRAP1, SYNPO2, FHF2, NCAN, CRP, WWC3, GAN, DPY19L3, DHCR7, OSBL1, SLC22A1, OMD, DPT, HLA-DRB3, NTS, TRIM41, CCL17
De Novo Metastasis	Lower	ERAP2, COL9A1, RAVER1, EPHA3, MANEAL, MAPK13, SIGIRR, RPD3, HEMGN, BDH2, AKAP12, TMSB4X, LCMT1, CLEC14A, IGHV3-15, TBC1D30, TRAPPC2, TRAPPC2B, WDR41, GXAL, TTN, DST, TMEM131A, CKM, KMT2B, IMY, IGHV3-21, DNAH6, COCH, ISL1, SPTB, WWC3, KRT19, IGKV2-29, IGKV3D-20, KISS1
Local relapse	Higher	GAN, CYB561D2, DLST, PRMT9, OGA, PSEN2, CNDP1, PPP6R3, DPP3, ESD, CDH1, DAG1, AUP1, PGAM5, DAD1, FCN1, FOL1, SEMG, PGAS, PGA4, PGA3, FMOD
Local relapse	Lower	ZNF428, HDGFL2, GALE, HNRNPL, LEUTX1, HNRNPA1, TBYN1, AHK5, LAMTOR3, MFGE8, CD69, REEP5, CHST7, ATP6V0A1, RDX, SLIT1, HLA-A, SESN3, SYNPO2, CDYBP2, SET, SELENOH, CCL19, HHAT, CLECO4, FZR, SLC9A1, SLC29A1, PRZH, SEPT10
Distant relapse	Higher	GASK1A, HDAC1, MARCHF2, TRIM28, TRAPPC11, ADAMTS7, HLA-DRB3, PKM, CXCL6, PRMT9, MROH1, MED25, NCAN, AMDHD1, POSK1, ARFGAP1, ZNHIT1, ZNF358, CBX1, LRP5, SYNPO2, HIVEP1, DEFA5, HLA-DPB1, DUSP7, STOXL3, GLS, DENND5B, AUP1, SLC17A5, SLC9A2
Distant relapse	Lower	GASK1A, ANKRD50A, SMEM15, HPO81, B4GALT5, ZNF846, PRF1, MINPP1, FSL, RALYL, EPB41, TMEM192D, NCF1B, IAG1, CLEC14A, GALC, SNRPB2, SLFN5, CALU, SLC12A4, CROCC, DSCAML1, IGHV3-35, TTN, IGHV1-39, CYP2C9, HSD11B1, COL2A1, STAU2
Neck Relapse	Higher	NID2, APP, XYLT2, GNPAT, MRPL17, CCNDBKN, CTSA, TNFSF12, SRGN, PROZ, CALU, C1S, FGL1, SOD3
Neck Relapse	Lower	SPHB1, CNBP, EMX2OS-4, LSM8, ABCB6, ANTXR2, CLEC3B, LIF, F11, CHQTN3, ANTXR1, PPP4R2A

[Fig. 2A]

Gene	Mutation type	Median (% in neoplastic cells) Relapse group	Non-relapse group	Wilcoxon test p value [<0.05]	Logistic regression Odds ratio	p value [<0.1]	Cox regression Hazard Ratio	p value [<=0.05]
HMGN2P3	SNV	0.392	0.000	<0.001	133796.394	0.052	66.335	0.005
DNAJC11	InDel	0.602	0.000	<0.001	79.707	0.018	13.422	0.003
EIF2AK1	InDel	0.445	0.000	0.004	30.381	0.030	8.126	0.031
FAM234A	InDel	0.628	0.000	0.013	6.666	0.015	8.102	0.002
PARPBP	InDel	0.366	0.000	0.002	13.734	0.034	6.358	0.023
ARL5A	SNV	0.314	0.000	0.004	6.525	0.016	5.219	0.002
IL32	SNV	0.800	0.000	0.001	2.779	0.046	2.209	0.051
DHX57	SNV	0.157	0.000	<0.001	120160606.586	0.066	1.704	0.006

[Fig. 2B]

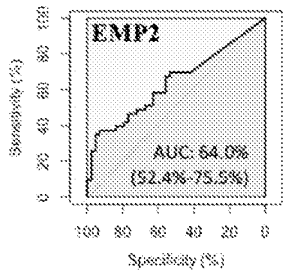
Gene	Detection threshold	Sensitivity (%)	Specificity (%)	Accuracy (%)
IL32	0.10	80	86.8	86.0
HMGN2P3	0.29	60	100.0	95.3
DHX57	0.16	60	100.0	95.3
DNAJC11	0.30	60	97.4	93.0
PARPBP	0.31	60	94.7	90.7
EIF2AK1	0.38	60	94.7	90.7
FAM234A	0.58	60	89.5	86.0
ARL5A	0.16	60	89.5	86.0

[Fig. 3A]

Gene	Mutation type	p value (wilcoxon test)	Sensitivity [>=40] (%)	Specificity (%)	EBV-ve [DNA <=50 copy/ml] coverage (%)
EMP2	InDel	0.008609945	69.8	41.9	66.7
IL32	InDel	1.88E-05	55.8	62.8	33.3
EIF2KMT	SNV	0.001758873	48.8	74.4	25.0

[Fig. 3B]

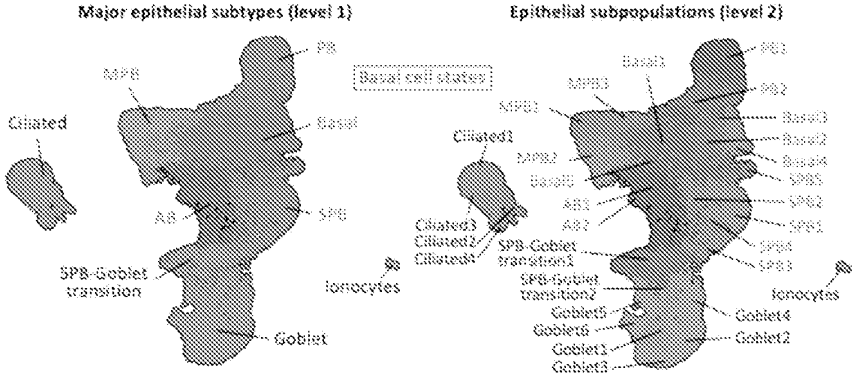
Detection threshold	Sensitivity (%)	Specificity (%)	Accuracy (%)
0.03	69.8	41.9	55.8
0.08	69.8	44.2	57.0
0.16	69.8	46.5	58.1
0.26	69.8	48.8	59.3
0.58	69.8	51.2	60.5
0.92	69.8	53.5	61.6



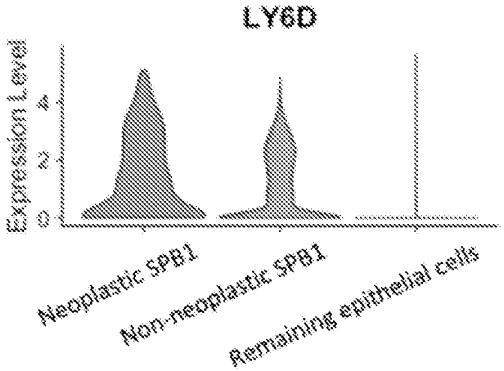
[Fig. 3C]

Mutation panel	Sensitivity (%)	EBV-ve [DNA <=50 copy/ml] coverage (%)
EMP2+IL32+EEF2KMT+CSTA+SOCS1+TESMIN+IGFBP7	93.0	83.3
EBV alone	72.1	NA
EMP2+IL32+EEF2KMT+CSTA+SOCS1+TESMIN+IGFBP7 + EBV	95.3	83.3

[Fig. 4A]



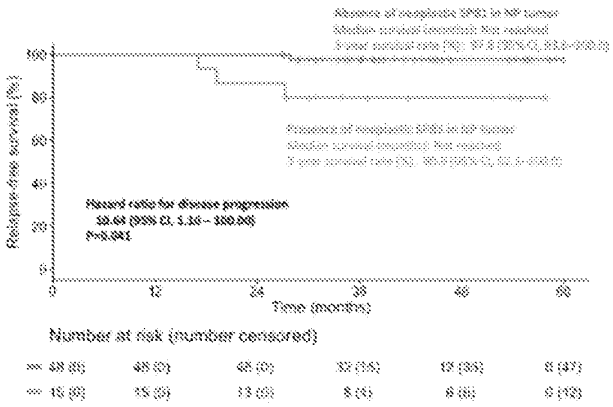
[Fig. 4B]



[Fig. 4C]

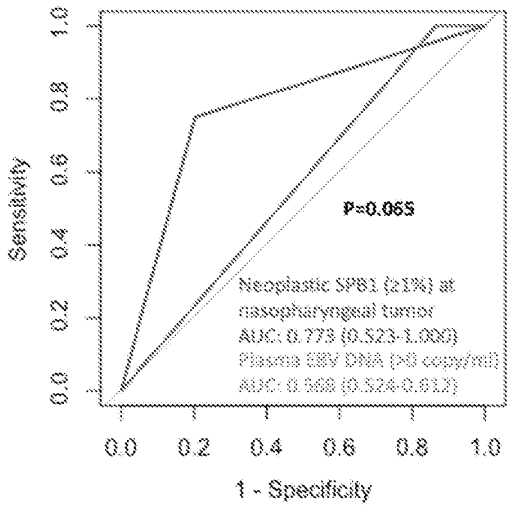
Site: NP Tumor	Median		Wilcoxon Test
Neoplastic Subpopulation	Relapse (%)	No Relapse (%)	P Value
SPB2	2.2	0.0	0.005
SPB5	0.7	0.0	0.013
SPB1	2.3	0.0	0.034
MPB2	0.1	0.0	0.039
MPB1	0.6	0.0	0.083
Basal2	12.8	21.0	0.111
PB1	19.8	8.3	0.145
Basal4	2.5	0.0	0.194
Basal1	28.5	18.4	0.209
SPB3	0.0	0.0	0.361
SPB4	1.1	0.0	0.409
MPB3	0.0	0.0	0.511
AB2	0.1	0.0	0.528
AB1	3.8	5.1	0.660
Basal5	0.6	0.3	0.720
Goblet4	0.0	0.0	0.745
Goblet6	0.0	0.0	0.845
Goblet1	0.0	0.0	0.845
SPB-Goblet transition1	0.0	0.0	0.845
PB2	10.1	12.4	0.899
Basal3	0.3	0.0	1.000

[Fig. 4D]



Neoplastic Subpopulations in NP Tumor	Log-rank Test	P Value
SPB5	3.383	0.066
SPB2	3.032	0.025
SPB1	2.120	0.086
Basal4	1.713	0.191
AB1	1.198	0.275
PB1	1.093	0.296
PB2	1.083	0.298
MPB2	1.014	0.314
Basal1	0.882	0.348
AB2	0.650	0.420
SPB4	0.608	0.435
Basal2	0.554	0.457
MPB1	0.519	0.471
SPB3	0.330	0.566
Basal3	0.193	0.660
Basal4	0.172	0.679
Goblet4	0.148	0.701
MPB3	0.148	0.701
Goblet1	0.040	0.842
Goblet6	0.040	0.842

[Fig. 4E]

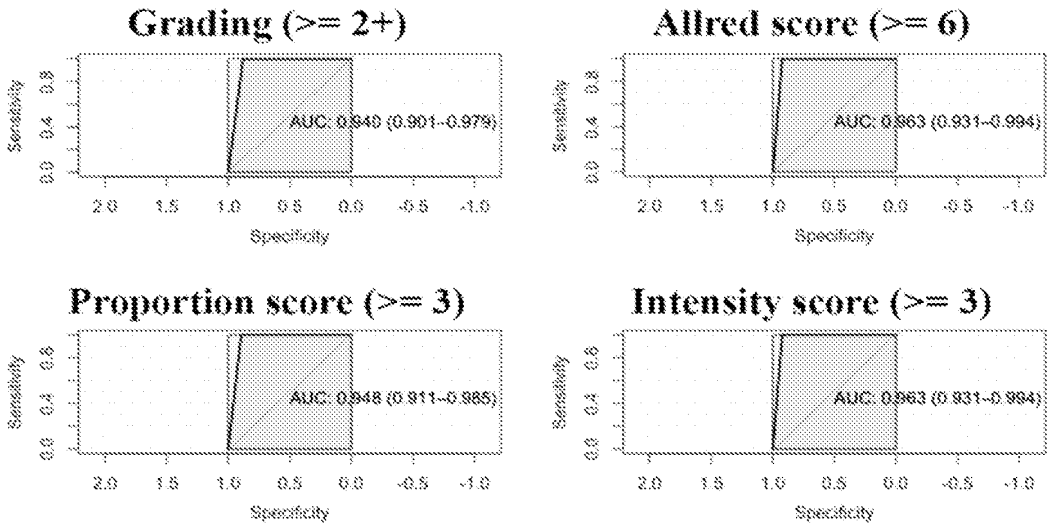


Threshold	Specificity	Sensitivity (>= 75%)	Accuracy
1.27%	81.4%	75.0%	81.0%

[Fig. 5]

ATPAF1 chr1:46668177 [A>C]	Sensitivity (%)	Specificity (%)	Accuracy (%)	PPV (%)
Neoplastic SPB1	80.0	98.0	96.4	80.0
Neoplastic cells	100.0	84.3	85.7	38.5
Neoplastic cells excluding SPB1	100.0	84.3	85.7	38.5
Epithelial cells	100.0	78.4	80.4	31.3
Non-neoplastic cells	80.0	80.4	80.4	28.6

[Fig. 6]



Scoring	Cut-off	Specificity	Sensitivity	Accuracy
Intensity_score	>=3	92.5%	100.0%	93.1%
Allred_score	>=6	92.5%	100.0%	93.1%
Proportion_score	>=3	89.6%	100.0%	90.3%
Grade	>= 2+	88.1%	100.0%	88.9%

[Fig. 7A]

Gene	Threshold of detection	Sensitivity (%)	Specificity (%)	Accuracy (%)	Cell surface (GO:0009986)	Extracellular space (GO:0005615)	Median (% in neoplastic cells) Relapse group (1)	No relapse group (0)	Difference (1-0)	Wilcoxon test p value [<0.05]	Logistic regression Odds ratio	p value [<0.05]	Cox regression Hazard ratio	p value [<0.1]
NEDD8	1.24	100.0	91.0	91.5	No	**Yes**	1.31	1.04	0.27	0.003	63445.2	0.011	1622.9	0.009
CALML3	1.57	100.0	88.1	88.7	No	**Yes**	1.68	0.70	0.98	0.007	4.0	0.023	4.2	0.019
GNG5	1.81	75.0	97.0	95.8	No	**Yes**	1.83	1.49	0.34	0.002	774213087.2	0.031	23469.7	0.006
UBE2D2	1.19	75.0	97.0	95.8	No	**Yes**	1.22	0.91	0.32	0.003	1511516.5	0.014	76812.6	0.009
PSMA4	1.24	75.0	91.0	90.1	No	**Yes**	1.25	0.97	0.29	0.006	1108.1	0.029	1906.4	0.018
SLC2A1	0.64	75.0	89.6	88.7	No	**Yes**	0.69	0.36	0.33	0.033	133.3	0.029	94.7	0.020
SNX3	1.18	75.0	89.6	88.7	No	**Yes**	1.25	0.96	0.29	0.045	77.3	0.018	17.0	0.064
LY6D	1.82	75.0	89.6	88.7	**Yes**	No	2.03	0.51	1.53	0.042	3.4	0.029	2.2	0.065
PSMA3	1.02	75.0	86.6	85.9	No	**Yes**	1.10	0.80	0.30	0.014	1054.7	0.019	1989.2	0.023
PSMB1	1.59	75.0	86.6	85.9	No	**Yes**	1.60	1.37	0.23	0.011	1036.4	0.025	751.7	0.020
YBX1	2.34	75.0	86.6	85.9	No	**Yes**	2.37	2.13	0.24	0.040	157.2	0.050	412.2	0.049
PSMB5	1.13	75.0	85.1	84.5	No	**Yes**	1.20	0.90	0.30	0.012	608.5	0.029	131.8	0.022
PSMA6	1.33	75.0	83.6	83.1	No	**Yes**	1.51	1.09	0.43	0.035	95.2	0.019	34.8	0.024

[Fig. 7B]

Gene	Threshold of detection	Sensitivity (%)	Specificity (%)	Accuracy (%)	Cell surface (GO:0009986)	Extracellular space (GO:0005615)
NDUFA13	1.44	100.0	92.5	93.0	No	No
BEX3	1.30	100.0	91.0	91.5	No	No
HNRNPA0	1.38	100.0	85.1	85.9	No	No
SLIRP	1.04	100.0	85.1	85.9	No	No
ADH5	1.13	100.0	80.6	81.7	No	Yes

[Fig. 8A]

Gene	Threshold of detection	Sensitivity (%)	Specificity (%)	Accuracy (%)	Cell surface (GO:0009986)	Extracellular space (GO:0005615)	Median (Expression) Neoplastic (1)	Non-neoplastic (0)	Difference (1-0)	Wilcoxon test p value [<0.05]	Logistic regression Odds ratio	p value [<0.05]
CKAP4	0.57	94.2	92.5	93.3	No	Yes	1.0	0.4	0.5	<0.001	48966.7	<0.001
SYNGR2	1.50	92.3	96.2	94.3	No	Yes	2.0	1.3	0.7	<0.001	179.9	<0.001
MARCKSL1	1.03	92.3	90.6	91.4	No	Yes	1.8	0.8	1.0	<0.001	50.6	<0.001
CFL1	2.08	90.4	98.1	94.3	No	Yes	2.5	1.8	0.7	<0.001	162.3	<0.001
PDCD5	0.95	90.4	94.3	92.4	No	Yes	1.3	0.8	0.5	<0.001	1743.9	<0.001
IL32	1.19	88.5	92.5	90.5	No	Yes	2.1	0.6	1.4	<0.001	25.1	<0.001
RAN	1.64	88.5	92.5	90.5	No	Yes	2.1	1.3	0.8	<0.001	68.4	<0.001
NME1	0.66	88.5	90.6	89.5	No	Yes	1.0	0.5	0.5	<0.001	574.3	<0.001
VCAM1	0.26	88.5	90.6	89.5	Yes	Yes	0.8	0.1	0.8	<0.001	4467.6	<0.001
GAPDH	3.72	86.5	98.1	92.4	No	Yes	4.3	3.3	1.0	<0.001	29.1	<0.001
TUBB	1.83	86.5	98.1	92.4	No	Yes	2.4	1.3	1.1	<0.001	20.6	<0.001
HSPD1	1.42	86.5	90.6	88.6	Yes	Yes	1.8	1.2	0.6	<0.001	73.1	<0.001
LGALS1	0.75	86.5	90.6	88.6	No	Yes	1.8	0.3	1.5	<0.001	20.2	<0.001
TNFAIP3	0.52	86.5	90.6	88.6	No	Yes	0.9	0.3	0.6	<0.001	54.1	<0.001
ITGAV	0.48	86.5	88.7	87.6	Yes	Yes	0.8	0.3	0.5	<0.001	795.1	<0.001
RPLP0	3.98	84.6	96.2	90.5	No	Yes	4.3	3.7	0.7	<0.001	27.4	<0.001
STMN1	0.80	84.6	88.7	86.7	No	Yes	1.4	0.4	1.0	<0.001	43.5	<0.001
PPIA	3.12	82.7	98.1	90.5	No	Yes	3.5	2.7	0.8	<0.001	46.7	<0.001
CCL20	1.01	82.7	96.2	89.5	No	Yes	2.0	0.2	1.9	<0.001	16.3	<0.001
FSCN1	0.42	82.7	94.3	88.6	No	Yes	0.8	0.2	0.6	<0.001	1077.5	<0.001
LGALS9	0.62	82.7	84.9	83.8	No	Yes	1.1	0.5	0.6	<0.001	34.7	<0.001
RPS19	3.83	80.8	98.1	89.5	No	Yes	4.1	3.5	0.6	<0.001	51.2	<0.001
YBX1	2.05	80.8	98.1	89.5	No	Yes	2.3	1.7	0.5	<0.001	42.6	<0.001
TPI1	2.05	80.8	96.2	88.6	No	Yes	2.3	1.8	0.5	<0.001	55.4	<0.001
ICAM1	0.47	78.8	98.1	88.6	Yes	Yes	0.8	0.2	0.6	<0.001	420.3	<0.001
ENO1	1.70	78.8	92.5	85.7	Yes	Yes	2.1	1.4	0.6	<0.001	44.9	<0.001
C1QBP	1.28	76.9	98.1	87.6	Yes	Yes	1.5	1.0	0.5	<0.001	93.6	<0.001
CXCL3	0.38	76.9	92.5	84.8	No	Yes	0.6	0.1	0.5	<0.001	410.1	<0.001
MIF	2.58	73.1	98.1	85.7	Yes	Yes	3.0	2.2	0.8	<0.001	6.6	<0.001
TAGLN2	2.15	73.1	88.7	81.0	No	Yes	2.4	1.9	0.5	<0.001	7.8	<0.001
CXCL10	1.26	71.2	90.6	81.0	Yes	Yes	1.8	0.3	1.5	<0.001	6.4	<0.001

[Fig. 8B]

Gene	Extracellular region (GO:0005576)	Median Expression			Wilcoxon Test
		Neoplastic (1)	Non-neoplastic (0)	Difference(1-0)	P Value
CCL20	Yes	2.04	0.16	1.88	<0.001
LGALS1	Yes	1.79	0.31	1.48	<0.001
CXCL10	Yes	1.75	0.29	1.47	<0.001
IL32	Yes	2.06	0.64	1.42	<0.001
UBD	No	2.53	1.37	1.17	<0.001
TUBB	Yes	2.38	1.31	1.06	<0.001
CSTA	Yes	2.24	1.23	1.02	<0.001
NFKBIA	No	2.00	1.00	1.01	<0.001
MARCKSL1	Yes	1.77	0.78	1.00	<0.001
STMN1	Yes	1.43	0.44	0.99	<0.001
SOCS1	No	1.14	0.16	0.99	<0.001
GAPDH	Yes	4.28	3.32	0.95	<0.001
MYBPC1	No	0.92	0.04	0.88	<0.001
NTRK2	No	1.04	0.19	0.85	<0.001
FKBP1A	No	1.81	0.97	0.84	<0.001
MIF	Yes	3.03	2.19	0.83	<0.001
SOX4	No	2.01	1.18	0.83	<0.001
RAN	Yes	2.08	1.26	0.83	<0.001
TUBA1C	No	1.67	0.87	0.81	<0.001
PPIA	Yes	3.47	2.67	0.80	<0.001
PYCARD	Yes	1.75	0.96	0.79	<0.001
VCAM1	Yes	0.84	0.06	0.78	<0.001
TCIM	No	1.01	0.24	0.78	<0.001

[Fig. 8C]

Gene	Extracellular region (GO:0005576)	Median Expression			Wilcoxon Test
		Neoplastic (1)	Non-neoplastic (0)	Difference(1-0)	P Value
SLPI	Yes	0.29	4.09	-3.79	<0.001
WFDC2	Yes	1.12	4.08	-2.96	<0.001
AQP3	No	0.25	3.05	-2.80	<0.001
LCN2	Yes	0.08	2.65	-2.57	<0.001
BPIFB1	Yes	0.00	2.43	-2.43	<0.001
SERPINB3	Yes	0.01	2.42	-2.41	<0.001
CLU	Yes	0.55	2.95	-2.41	<0.001
SCGB1A1	Yes	0.00	2.34	-2.33	<0.001
CRIP1	No	0.42	2.41	-1.99	<0.001
KRT14	Yes	0.11	2.05	-1.94	<0.001
LYPD2	Yes	0.00	1.90	-1.90	<0.001
TFF3	Yes	0.06	1.96	-1.90	<0.001
TSPAN1	Yes	0.15	2.01	-1.86	<0.001
SCGB3A1	Yes	0.00	1.82	-1.82	<0.001
PIGR	Yes	0.00	1.78	-1.78	<0.001
MUC16	Yes	0.08	1.82	-1.73	<0.001
C19orf33	No	0.07	1.80	-1.73	<0.001
PRSS23	Yes	0.17	1.89	-1.72	<0.001
MSMB	Yes	0.15	1.84	-1.69	<0.001
CAPS	No	0.06	1.72	-1.66	<0.001
CXCL17	Yes	0.10	1.75	-1.65	<0.001
ANXA1	Yes	0.89	2.53	-1.63	<0.001
AGR2	Yes	0.49	2.11	-1.62	<0.001
GSTA1	Yes	0.37	1.97	-1.61	<0.001
BPIFA1	Yes	0.00	1.54	-1.54	<0.001
MT1X	No	0.61	2.12	-1.51	<0.001
ALDH3A1	Yes	0.19	1.69	-1.50	<0.001
C20orf85	No	0.00	1.44	-1.44	<0.001
LGALS3	Yes	1.18	2.60	-1.42	<0.001
MUC4	Yes	0.05	1.45	-1.40	<0.001
TACSTD2	Yes	1.22	2.61	-1.39	<0.001

Biomarker combination	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
$CCL20_{high} + IL32_{high} + LCN2_{low}$	100.0	96.2	98.1	96.3	100.0

[Fig. 9A]

EBV transcripts detected

Virus	Gene	Sensitivity (%)
Human gammaherpesvirus 4	BPMS1	100.0
Human gammaherpesvirus 4	LMP-1	91.0
Human gammaherpesvirus 4	LMP-2B	76.1
Human gammaherpesvirus 4	A73	64.2
Human gammaherpesvirus 4	LF1	55.2
Human gammaherpesvirus 4	BALF5	49.3
Human gammaherpesvirus 4	BNRF1	49.3
Human gammaherpesvirus 4	BILF1	44.8
Human gammaherpesvirus 4	BARF0	41.8
Human gammaherpesvirus 4	EBNA-1.2	31.3
Human gammaherpesvirus 4	BALF4	14.9
Human gammaherpesvirus 4	EBNA-3B/EBNA-3C	14.9
Human gammaherpesvirus 4	BKLF1	13.4
Human gammaherpesvirus 4	BALF2	9.0
Human gammaherpesvirus 4	BMRF2	7.5
Human gammaherpesvirus 4	BPLF1	7.5
Human gammaherpesvirus 4	EBNA-3A	7.5
Human gammaherpesvirus 4	EBNA-2	6.0
Human gammaherpesvirus 4	LF2	6.0
Human gammaherpesvirus 4	BALF3	4.5
Human gammaherpesvirus 4	BBRF3	4.5
Human gammaherpesvirus 4	BCRF1.1	4.5
Human gammaherpesvirus 4	BLRF2	4.5
Human gammaherpesvirus 4	BORF2	4.5
Human gammaherpesvirus 4	BBLF4	1.5
Human gammaherpesvirus 4	BBRF1	1.5
Human gammaherpesvirus 4	BGLF2	1.5
Human gammaherpesvirus 4	BGRF1/BDRF1	1.5
Human gammaherpesvirus 4	BHLF1	1.5
Human gammaherpesvirus 4	BKRF3	1.5
Human gammaherpesvirus 4	BSLF1	1.5
Human gammaherpesvirus 4	BTRF1	1.5
Human gammaherpesvirus 4	BVLF1	1.5
Human gammaherpesvirus 4	BVRF1	1.5
Human gammaherpesvirus 4	BZLF2	1.5
Human gammaherpesvirus 4	LMP-2A	1.5

EBV detection method	Sensitivity (%)
EBV transcript at NP tumour	100.0
Plasma EBV DNA (>0 copy number/ml = positive)	87.5

[Fig. 9B]

Significant EBV Transcript for NPC Relapse Prediction (Tumor Tissue Sample)

	Logistic Regression Test		Cox Regression Test	
EBV Gene	odds_ratio	p_value	HR	p_value
NC_007605.1_BARF0.gene.HHV4_BARF0	64.00	0.001	40.49	0.001
NC_007605.1_BALF4.gene.HHV4_BALF4	25.71	0.008	18.24	0.012
NC_007605.1_RPMS1.gene.HHV4_RPMS1	25.71	0.008	21.61	0.008
NC_007605.1_EBNA.3A.gene.HHV4_EBNA.3A	22.00	0.044	19.99	0.017
NC_007605.1_EBNA.3B.EBNA.3C.gene.HHV4_EBNA.3B.EBNA.3C	22.00	0.044	19.99	0.017

Table 1 significant EBV transcripts associated to NPC relapse in tumor tissue samples(logistic and cox regression test)

Significant EBV Transcript for NPC Relapse Prediction
(Lymph Node Tissue Sample)

	Cox Regression Test	
EBV Gene	HR	p_value
NC_007605.1_LF2.gene.HHV4_LF2	18.97	0.037

Table 2. significant EBV transcripts associated to NPC relapse in lymph node tissue samples(cox regression test)

Significant EBV Transcript for NPC Relapse Prediction (Tumor and Lymph Node Tissue Sample)

	Logistic Regression Test		Cox Regression Test	
EBV Gene	odds_ratio	p_value	HR	p_value
NC_007605.1_RPMS1.gene.HHV4_RPMS1	30.00	0.004	26.47	0.004
NC_007605.1_BALF4.gene.HHV4_BALF4	30.00	0.004	20.94	0.007
NC_007605.1_BARF0.gene.HHV4_BARF0	18.67	0.012	12.93	0.022
NC_007605.1_BALF5.gene.HHV4_BALF5	14.13	0.022	12.18	0.025

Table 3. significant EBV transcripts associated to NPC relapse in tumor and lymph node samples(logistic and cox regression test)

EBV Transcript Sensitivity For NPC Relapse Prediction

EBV Gene	True Positive Rate	False Positive Rate
NC_007605.1_BALF4.gene.HHV4_BALF4	0.8	0.12
NC_007605.1_RPMS1.gene.HHV4_RPMS1	0.8	0.12
NC_007605.1_BARF0.gene.HHV4_BARF0	0.8	0.18
NC_007605.1_BALF5.gene.HHV4_BALF5	0.8	0.22
NC_007605.1_BILF1.gene.HHV4_BILF1	0.8	0.43
NC_007605.1_A73.gene.HHV4_A73	0.8	0.46
NC_007605.1_LMP1.gene.HHV4_LMP1	0.8	0.47
NC_007605.1_LMP2B.gene.HHV4_LMP2B	0.8	0.47

Table 4. EBV transcript sensitivity test for NPC Relapse Prediction

Gene candidates
NC_007605.1_BALF4.gene.HHV4_BALF4
NC_007605.1_RPMS1.gene.HHV4_RPMS1
NC_007605.1_BARF0.gene.HHV4_BARF0
NC_007605.1_BALF5.gene.HHV4_BALF5

These four EBV genes(transcripts) were consistently found to be associated with relapse and have sensitivity of at least 0.8 and low false positive rate

[Fig. 10A]

Abundance estimation of epithelial subpopulations using the signature matrix specified in FIG. 13A and association with relapse prediction in validation cohort GSE182349 by machine learning

Site: NP tumour	Median			Wilcoxon test
Epithelial subpopulation	Relapse group	No relapse group	Relapse-to-no relapse ratio	p value
Neoplastic PB1	3.367	0.304	11.091	0.001
Non-neoplastic AB2	3.424	12.260	0.279	0.003
Non-neoplastic Goblet6	0.259	0.000	NE	0.007
Neoplastic Basal2	0.020	0.001	NE	0.009
Neoplastic SPB1	0.958	0.024	40.737	0.011
Non-neoplastic Goblet1	0.000	0.385	0.000	0.020
Neoplastic AB3	3.892	8.689	0.448	0.024
Non-neoplastic Ciliated1	0.172	0.580	0.296	0.038
Non-neoplastic SPB1	0.149	0.958	0.157	0.048
Non-neoplastic Basal1	0.000	0.000	NE	0.057
Neoplastic Basal5	0.217	0.000	NE	0.061
Neoplastic MPB1	0.790	1.752	0.451	0.067
Neoplastic SPB5	0.005	0.437	0.011	0.081
Non-neoplastic Basal3	0.069	0.612	0.112	0.109
Neoplastic MPB3	0.000	0.058	0.000	0.112
Non-neoplastic Basal2	0.076	0.000	NE	0.117
Neoplastic SPB2	0.000	0.629	0.000	0.131
Non-neoplastic SPB3	0.040	0.000	NE	0.147
Non-neoplastic PB2	0.665	0.419	1.587	0.148
Neoplastic PB2	2.603	1.744	1.527	0.184
Non-neoplastic Goblet4	0.096	0.015	0.000	0.173
Neoplastic SPB4	0.009	0.000	NE	0.196
Non-neoplastic Goblet2	0.014	0.031	0.447	0.213
Non-neoplastic Goblet5	0.008	0.000	NE	0.241
Non-neoplastic Basal5	0.000	0.001	NE	0.258
Non-neoplastic Ciliated4	0.000	0.000	NE	0.280
Non-neoplastic SPB-Goblet transition1	0.008	0.073	0.108	0.284
Non-neoplastic SPB2	0.000	0.001	NE	0.388
Non-neoplastic SPB-Goblet transition2	0.002	0.000	NE	0.427
Neoplastic Basal4	0.009	0.000	NE	0.491
Non-neoplastic MPB1	0.702	1.042	0.673	0.527
Non-neoplastic Iono cytes	0.009	0.000	NE	0.601
Non-neoplastic SPB5	0.000	0.006	NE	0.601
Neoplastic Basal3	0.000	0.000	NE	0.611
Non-neoplastic SPB4	0.307	0.325	0.944	0.614
Non-neoplastic Ciliated2	0.000	0.001	NE	0.685
Neoplastic MPB2	0.006	0.017	0.000	0.696
Non-neoplastic Ciliated3	0.013	0.007	1.821	0.705
Non-neoplastic Goblet3	0.021	0.003	6.476	0.737
Neoplastic SPB3	0.000	0.000	NE	0.740
Neoplastic Basal1	0.063	0.563	0.113	0.769
Non-neoplastic Basal4	0.582	0.870	0.669	0.775
Non-neoplastic PB1	0.397	0.081	4.885	0.890
Non-neoplastic MPB3	0.893	0.775	1.152	1.000

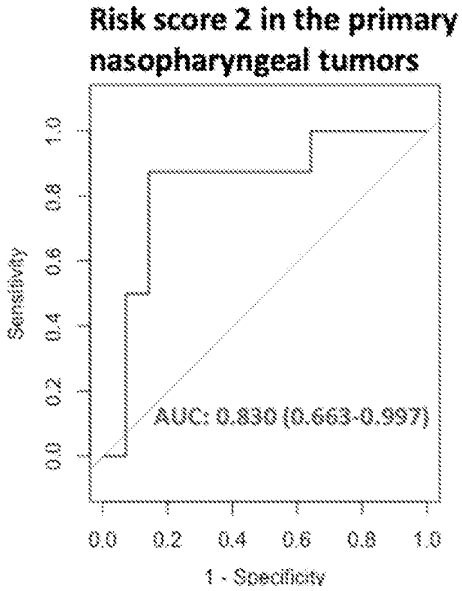
NE denotes not available

[Fig. 10B]

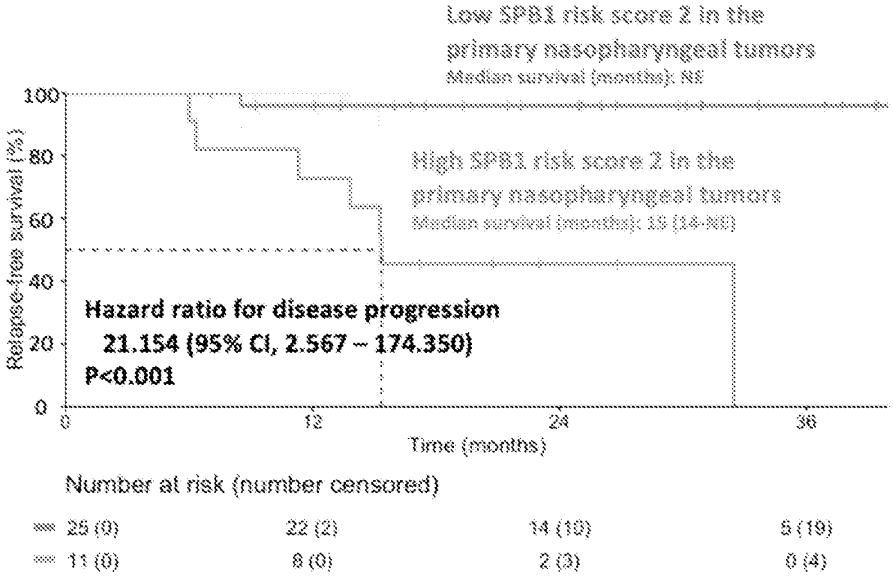
False positive prediction performance by non-neoplastic SPB1

Threshold (%)	Specificity (%)	Sensitivity (%)	Accuracy (%)
1.0	77.3	71.4	75.0
0.3	54.5	100.0	72.2

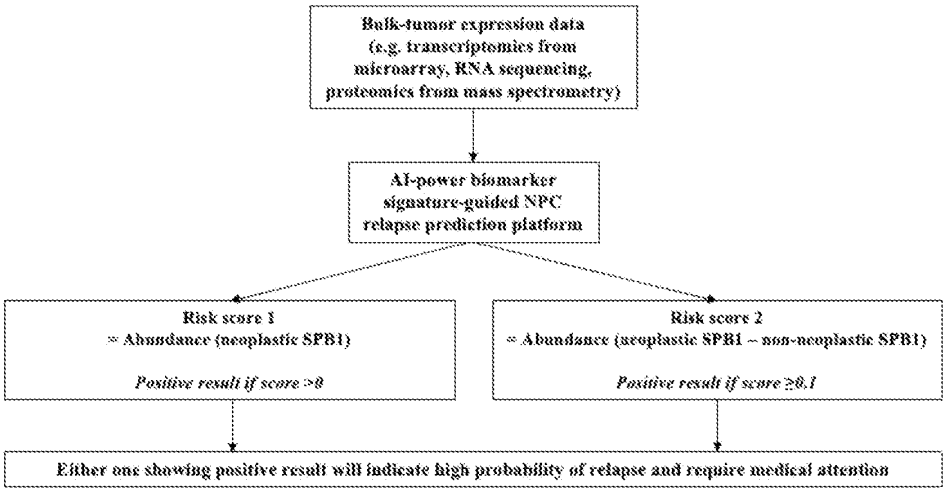
Risk score 2 = [Neoplastic SPB1 - Non-neoplastic SPB1 (%)]	Sensitivity (%)	Specificity (%)	Accuracy (%)
≥0.1	87.5	85.7	86.1
≥0.0	87.5	82.1	83.3



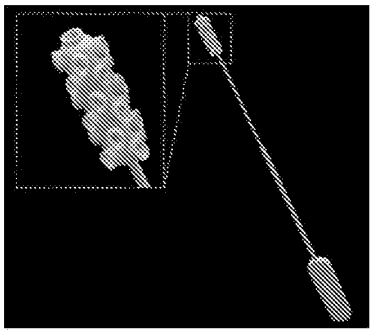
[Fig. 10C]



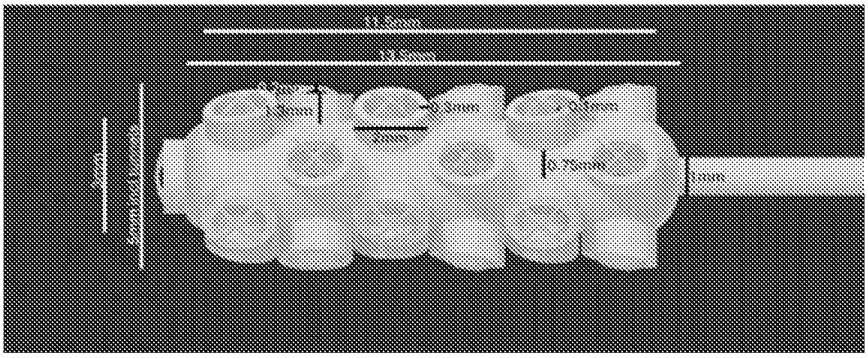
[Fig. 10D]



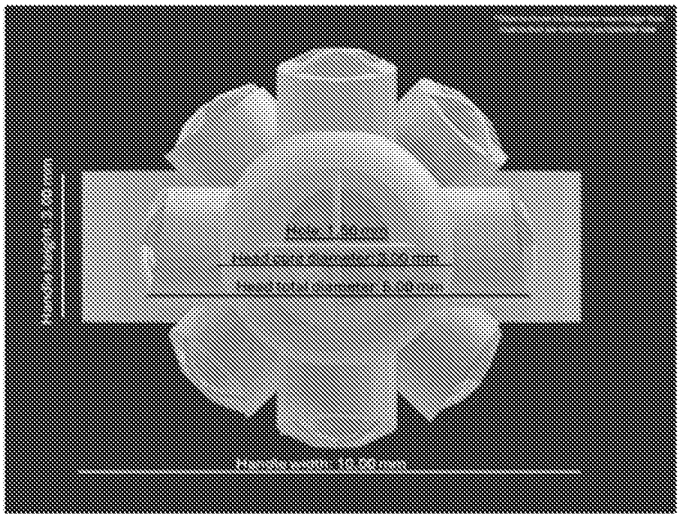
[Fig. 11A]



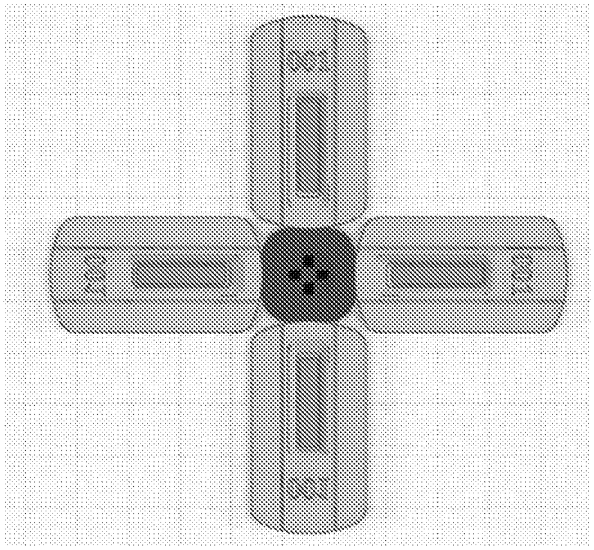
[Fig. 11B]



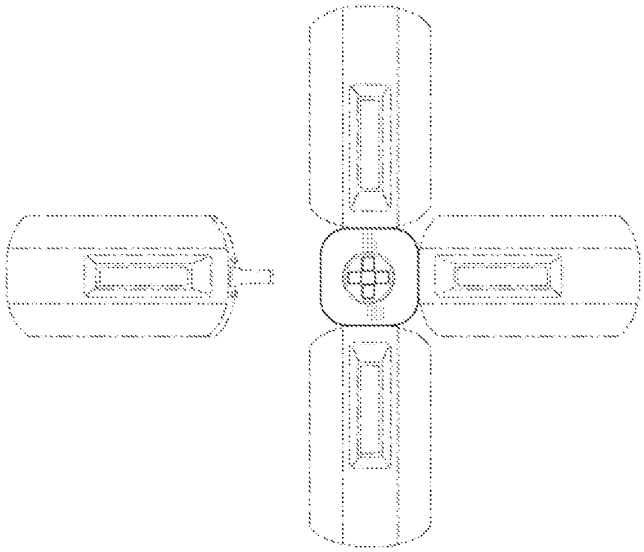
[Fig. 11C]



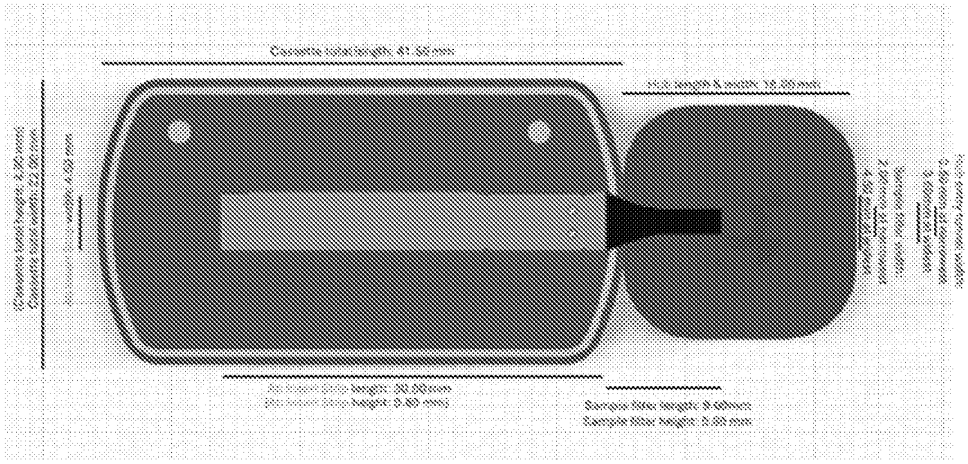
[Fig. 11D]



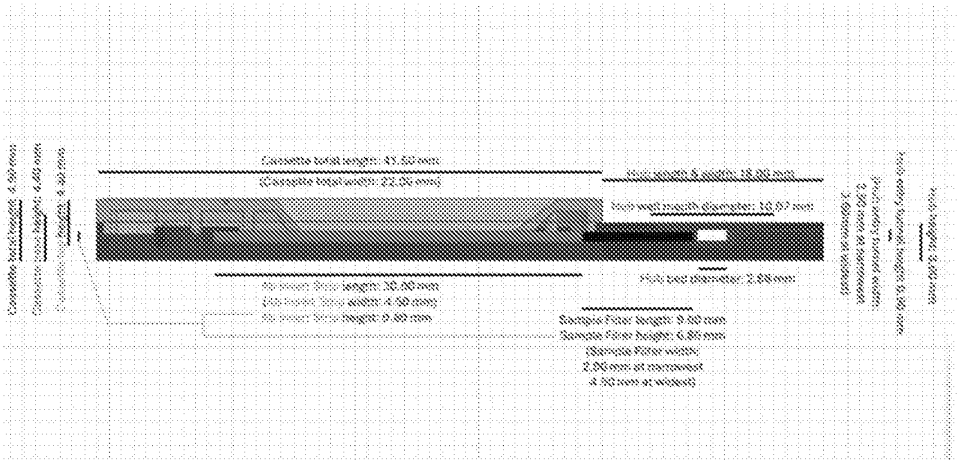
[Fig. 11E]



[Fig. 11F]

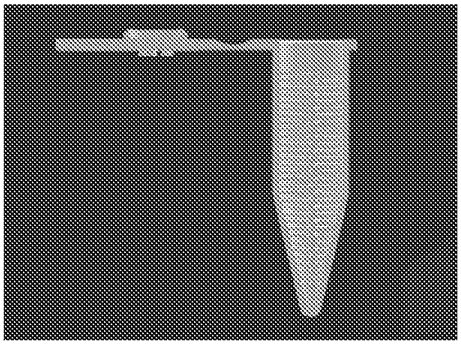


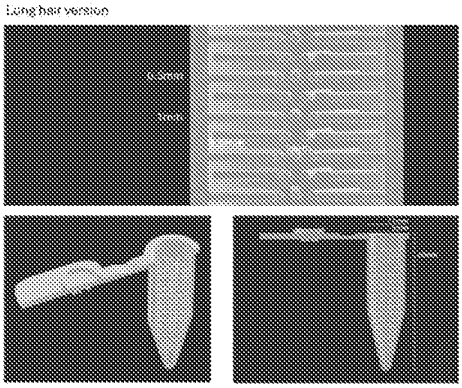
[Fig. 11G]



[Fig. 11H]

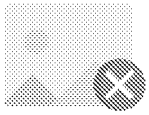
Rule 26, 10.01.2025





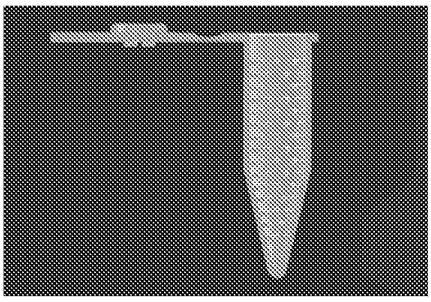
[Fig. 11H(C0nt'd)]

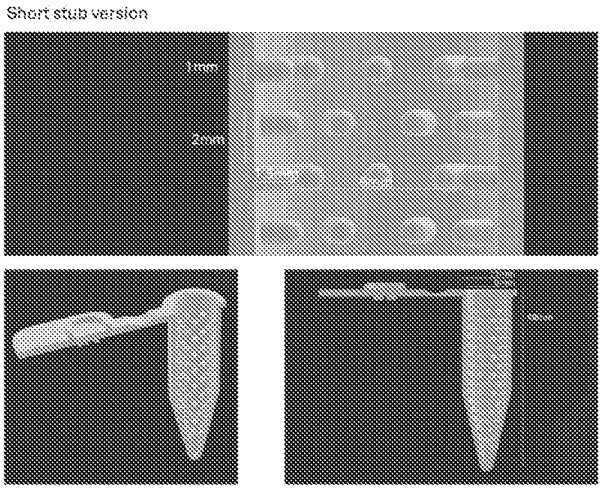
Rule 26, [Deleted,1



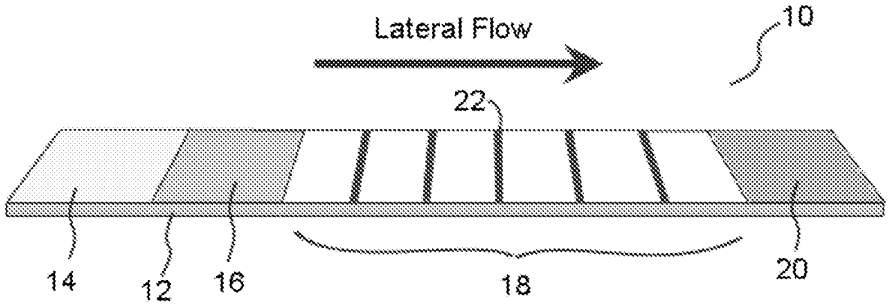
图片已删除

[Fig. 11I]

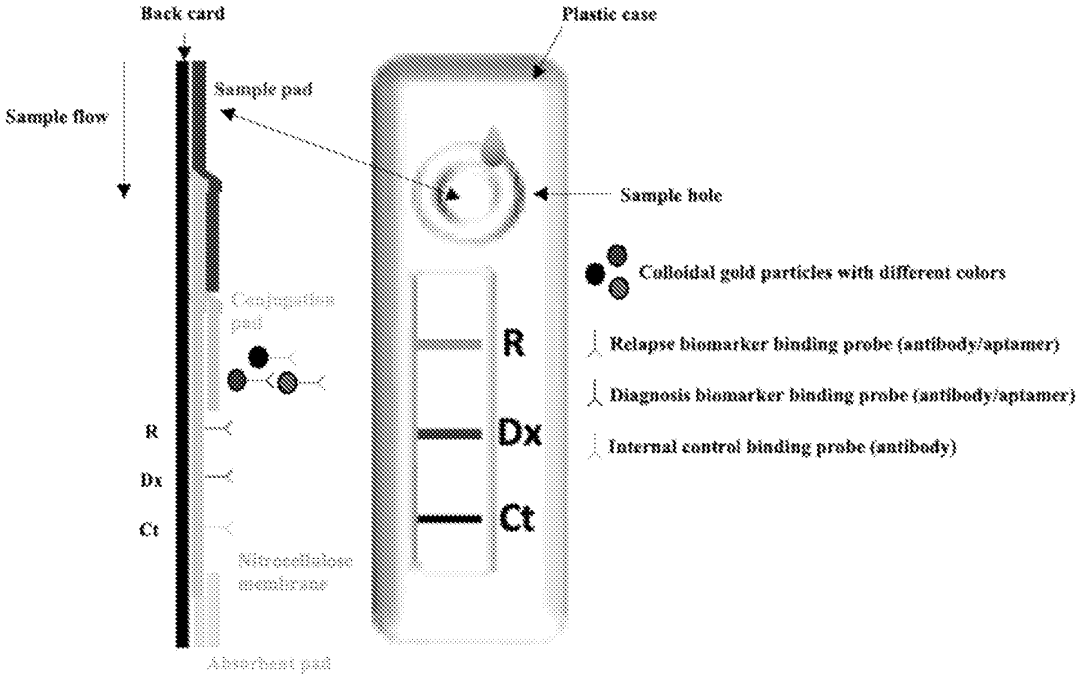




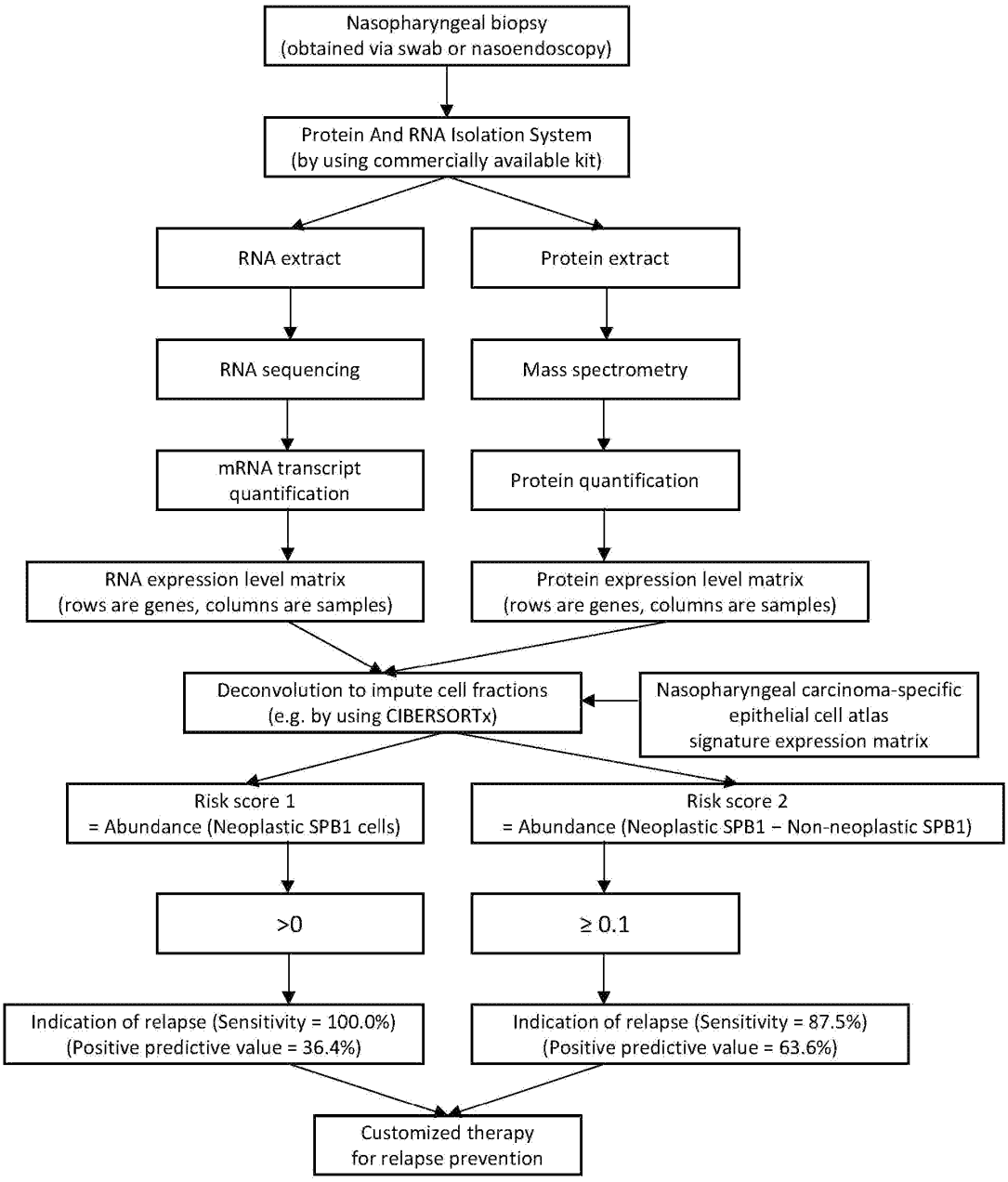
[Fig. 12A]



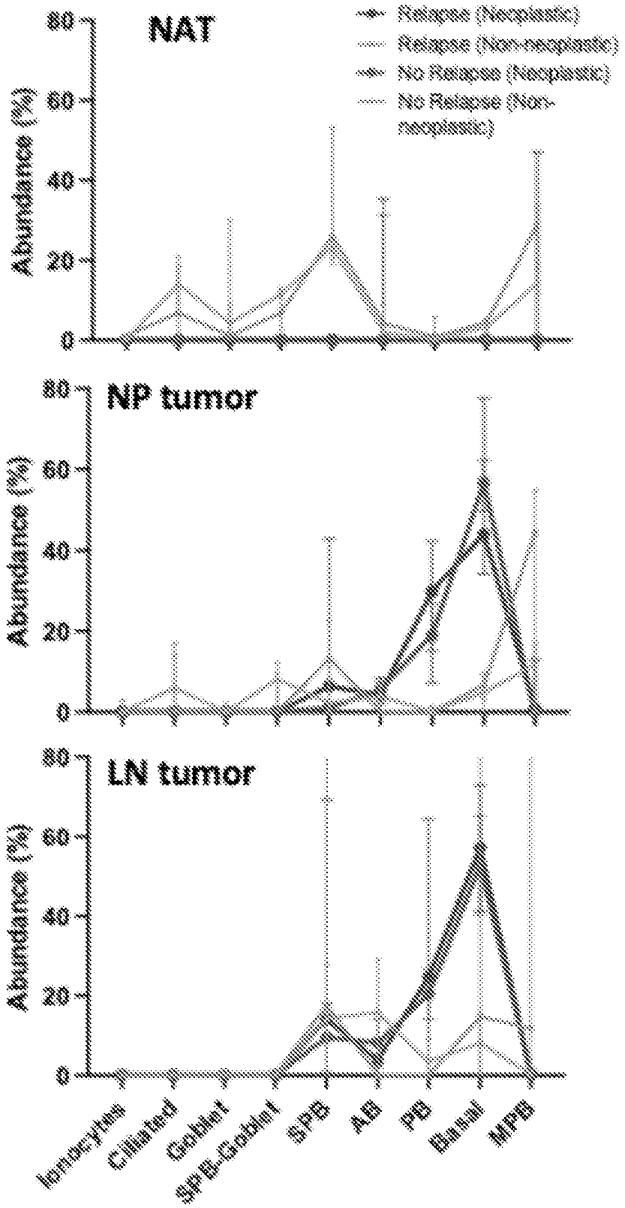
[Fig. 12B]


Back card
Plastic case
Sample flow
Sample pad
Sample hole
Colloidal gold particles with different colors
Conjugation pad
R
Relapse biomarker binding probe (antibody/aptamer)
Diagnosis biomarker binding probe (antibody/aptamer)
Dx
Internal control binding probe (antibody)
R
Dx
Ct
Ct
Nitrocellulose membrane
Absorbent pad

[Fig. 13]


Nasopharyngeal biopsy
(obtained via swab or nasoendoscopy)
Protein And RNA Isolation System
(by using commercially available kit)
RNA extract
Protein extract
RNA sequencing
Mass spectrometry
mRNA transcript
quantification
Protein quantification
RNA expression level matrix
(rows are genes, columns are samples)
Protein expression level matrix
(rows are genes, columns are samples)
Deconvolution to impute cell fractions
(e.g. by using CIBERSORTx)
Nasopharyngeal carcinoma-specific
epithelial cell atlas
signature expression matrix
Risk score 1
= Abundance (Neoplastic SPB1 cells)
Risk score 2
= Abundance (Neoplastic SPB1 − Non-neoplastic SPB1)
>0
≥ 0.1
Indication of relapse (Sensitivity = 100.0%)
(Positive predictive value = 36.4%)
Indication of relapse (Sensitivity = 87.5%)
(Positive predictive value = 63.6%)
Customized therapy
for relapse prevention

[Fig. 14A]


NAT
Relapse (Neoplastic)
Relapse (Non-neoplastic)
No Relapse (Neoplastic)
No Relapse (Non-neoplastic)
Abundance (%)
80
60
40
20
0
NP tumor
LN tumor
Ionocytes
Ciliated
Goblet
SPB-Goblet
SPB
AB
PB
Basal
MPB

[Fig. 14B]

NAT

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
Basal Relapse vs. No relapse	19.15	3.28 to 35.03	*	0.018
Ciliated Relapse vs. No relapse	-10.67	-26.55 to 5.20	ns	0.187
Goblet Relapse vs. No relapse	-8.44	-24.32 to 7.43	ns	0.296
Ionocytes Relapse vs. No relapse	-0.04	-15.91 to 15.84	ns	0.996

NP tumor

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
Basal Relapse vs. No relapse	15.62	1.25 to 29.99	*	0.033
Ciliated Relapse vs. No relapse	-6.95	-21.32 to 7.42	ns	0.342
Goblet Relapse vs. No relapse	-8.01	-22.38 to 6.36	ns	0.274
Ionocytes Relapse vs. No relapse	-0.66	-15.03 to 13.71	ns	0.928

Neck LN tumor

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
Basal Relapse vs. No relapse	0.69	-0.90 to 2.29	ns	0.387
Ciliated Relapse vs. No relapse	-0.04	-1.63 to 1.55	ns	0.959
Goblet Relapse vs. No relapse	-0.65	-2.24 to 0.94	ns	0.416
Ionocytes Relapse vs. No relapse	0.00	-1.59 to 1.59	ns	>0.999

NAT denotes normal tissue adjacent to the tumor. NP denotes nasopharynx. LN denotes lymph node.
* denotes P<0.05. ** denotes P<0.01. *** denotes P<0.001. **** denotes P<0.0001. ns denotes not significant.

[Fig. 14C]

NAT

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
AB				
Relapse vs. No relapse	1.45	-8.45 to 11.34	ns	0.774
Basal				
Relapse vs. No relapse	1.27	-8.63 to 11.16	ns	0.802
Ciliated				
Relapse vs. No relapse	-10.67	-20.57 to -0.78	*	0.035
Goblet				
Relapse vs. No relapse	-2.56	-12.45 to 7.34	ns	0.612
Ionocytes				
Relapse vs. No relapse	-0.04	-9.93 to 9.86	ns	0.994
MPB				
Relapse vs. No relapse	7.48	-2.42 to 17.37	ns	0.138
PB				
Relapse vs. No relapse	0.46	-9.44 to 10.35	ns	0.928
proliferating SPB				
Relapse vs. No relapse	1.05	-8.85 to 10.94	ns	0.835
SPB				
Relapse vs. No relapse	7.46	-2.44 to 17.36	ns	0.139
SPB-Goblet				
Relapse vs. No relapse	-5.89	-15.78 to 4.01	ns	0.243

NP Tumor

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
AB				
Relapse vs. No relapse	-5.21	-16.60 to 6.18	ns	0.370
Basal				
Relapse vs. No relapse	8.65	-2.75 to 20.04	ns	0.137
Ciliated				
Relapse vs. No relapse	-6.95	-18.34 to 4.44	ns	0.231
Goblet				
Relapse vs. No relapse	-3.62	-15.01 to 7.77	ns	0.533
Ionocytes				
Relapse vs. No relapse	-0.66	-12.05 to 10.73	ns	0.909
MPB				
Relapse vs. No relapse	-0.93	-12.32 to 10.46	ns	0.873
PB				
Relapse vs. No relapse	9.69	-1.70 to 21.08	ns	0.095
proliferating SPB				
Relapse vs. No relapse	1.60	-9.79 to 12.99	ns	0.782
SPB				
Relapse vs. No relapse	1.82	-9.57 to 13.21	ns	0.754
SPB-Goblet				
Relapse vs. No relapse	-4.39	-15.78 to 7.00	ns	0.450

Neck LN Tumor

2-way ANOVA	Predicted Mean Difference	95% CI of Difference	Significance	P Value
AB				
Relapse vs. No relapse	-7.35	-19.08 to 4.37	ns	0.218
Basal				
Relapse vs. No relapse	7.78	-3.94 to 19.51	ns	0.192
Ciliated				
Relapse vs. No relapse	-0.04	-11.77 to 11.69	ns	0.995
Goblet				
Relapse vs. No relapse	-0.28	-12.01 to 11.45	ns	0.963
MPB				
Relapse vs. No relapse	0.02	-11.71 to 11.74	ns	0.998
PB				
Relapse vs. No relapse	2.59	-9.14 to 14.31	ns	0.664
proliferating SPB				
Relapse vs. No relapse	-2.80	-14.52 to 8.93	ns	0.639
SPB				
Relapse vs. No relapse	0.46	-11.27 to 12.18	ns	0.939
SPB-Goblet				
Relapse vs. No relapse	-0.37	-12.10 to 11.35	ns	0.950

NAT denotes normal tissue adjacent to the tumor. NP denotes nasopharynx. LN denotes lymph node. ns denotes not significant

[Fig. 15]

Site: Lymph Node	Median		Wilcoxon Test	Cox Regression	
Neoplastic Subpopulation	Relapse (%)	No Relapse (%)	P Value	Hazard Ratio	P Value
MPB1	0.3	0.0	0.029	0.0	0.902
Basal3	0.3	0.0	0.072	1.2×10^{14}	0.297
Basal5	1.5	0.0	0.076	3.4×10^{3}	0.606
SPB2	0.8	0.0	0.180	8.1×10^{2}	0.096
MPB2	0.0	0.0	0.193	Infinty	0.999
AB2	0.0	0.0	0.294	0.0	0.999
SPB1	0.8	0.0	0.329	9.1×10^{1}	0.555
PB1	17.5	12.4	0.406	2.2×10^{2}	0.427
Basal2	16.5	20.9	0.426	0.0	0.292
Basal1	25.4	19.0	0.426	4.1	0.618
Basal4	0.7	0.0	0.704	5.5	0.839
SPB3	0.0	0.0	0.745	0.0	0.743
SPB4	0.3	0.0	0.745	0.0	0.720
Ciliated4	0.0	0.0	0.766	0.0	0.999
SPB-Goblet transition1	0.0	0.0	0.766	0.0	0.999
MPB3	0.0	0.0	0.766	0.0	0.999
Basal3	0.3	0.0	1.000	0.0	0.519
AB1	2.9	4.4	1.000	0.0	0.574
PB2	12.0	7.9	1.000	1.5×10^{2}	0.633
SPB5	0.0	0.0	1.000	0.0	0.782