The genomic, transcriptomic, an immunological profile of patients with recurrent/refractory NSCLC

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Background

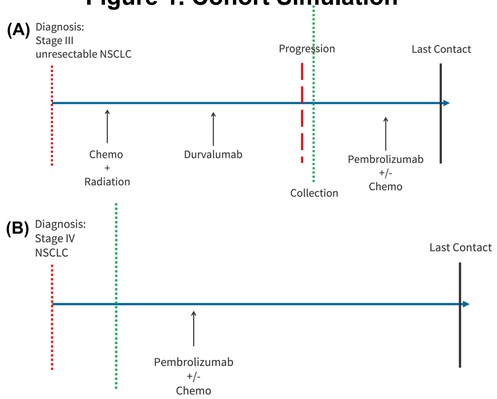
- Lung cancer is the leading cause of cancer-related deaths worldwide, and >50% of patients present as stage III or IV.
- Recently, patients with relapsing/remitting stage III NSCLC (R/R) were described to have worse overall survival compared to *de novo* stage IV (DN) patients when treated with chemoradiation and immunotherapy.
- In this study, we aimed to compare the molecular and immune landscapes of these two patient populations to identify potential differences in biomarkers of resistance and possible targets for therapy

Objectives and Methods

- 3728 NSCLC specimens underwent sequencing of DNA (592-gene panel or whole exome) or RNA (whole transcriptome) or immunohistochemistry at Caris Life Sciences (Phoenix, AZ).
- Patients were classified as R/R (n=26) if they received Durvalumab and chemoradiation within 12 months (m) before tissue collection and treated with Pembrolizumab within 6 m after tissue collection.
- Patients treated with Pembrolizumab within 6 months (m) after tissue collection but had no prior (or post) treatment with Durvalumab and chemoradiation at any point in time were classified as DN (n=3702).
- Tumor microenvironment (TME) cell fractions were estimated from bulk RNA sequencing using the QuanTIseq method.
- Statistical significance was determined using chi-square, Mann Whitney U and adjusted for multiple comparisons where applicable (q< 0.05).

Results

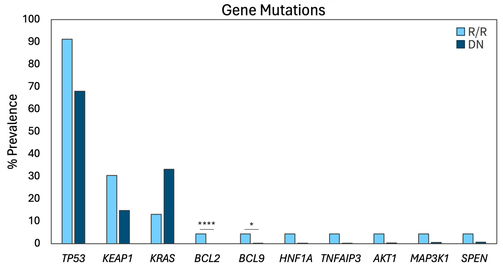
Figure 1. Cohort Simulation



(A) Patients with Stage 3 unresectable NSCLC are treated with chemoradiation + Durvalumab and upon progression are treated with Pembrolizumab, hence these specimens have previously been exposed to chemoradiation and Durvalumab
(B) Patients with Stage 4 (DN) are not exposed to chemoradiation and Durvalumab before treatment with Pembrolizumab.

These are the two populations our study is attempting to simulate and characterize

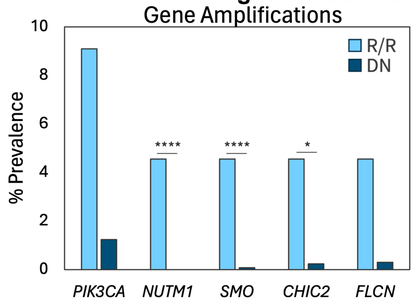
Figure 2. Mutational landscape associated with disease stage



Feature	# positive R/R Stage 3	# Total R/R Stage 3	% Prevalence R/R Stage 3	# positive de novo Stage 4	# Total de novo Stage 4	% Prevalence de novo Stage 4	p-value	q-value
TP53	21	23	91.3	1990	2924	68.06	0.0171	0.5683
KEAP1	7	23	30.43	439	2963	14.82	0.0363	0.7051
KRAS	3	23	13.04	983	2956	33.25	4.02E-02	0.7266
BCL2	1	23	4.35	0	2963	0	0.00E+00	0
BCL9	1	23	4.35	5	2962	0.17	0	0.0108
HNF1A	1	23	4.35	7	2964	0.24	0.0001	0.0542
TNFAIP3	1	23	4.35	7	2958	0.24	0.0001	0.0542
AKT1	1	23	4.35	11	2958	0.37	0.0027	0.259
MAP3K1	1	23	4.35	15	2868	0.52	0.0138	0.5289
SPEN	1	23	4.35	18	2955	0.61	0.0249	0.6433

Gene mutations highlighted had a statistical significance of p<0.05 between the two cohorts. Among the more prevalent mutations, mutations in *TP53* and *KEAP1* were higher, while mutations in *KRAS* were lower in R/R vs DN. Although at a low overall prevalence, mutations in BCL2 and BCL9 were higher in R/R vs DN (q<0.05). **** q<0.0001, *q<0.05

Figure 3. Gene amplifications associated with disease stage



Feature	# positive R/R Stage 3	# Total R/R Stage 3	% Prevalence R/R Stage 3	# positive de novo Stage 4	# Total de novo Stage 4	% Prevalence de novo Stage 4	p-value	q-value
PIK3CA	2	22	9.09	33	2651	1.24	0.0013	0.1767
NUTM1	1	22	4.55	0	2666	0	0	0
SMO	1	22	4.55	2	2668	0.07	0	0
CHIC2	1	22	4.55	6	2553	0.24	0.0001	0.0468
FLCN	1	22	4.55	8	2669	0.3	0.0006	0.1091

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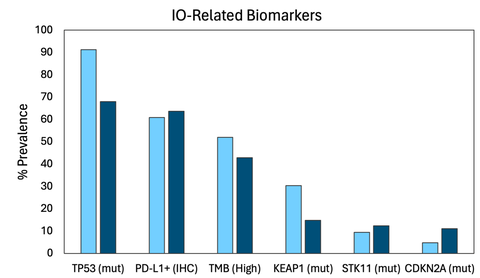
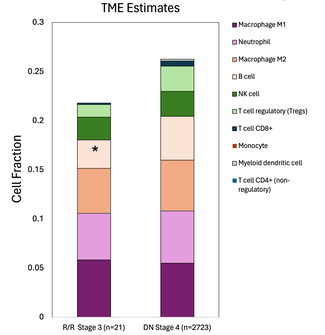
Figure 4. Association of IO-related biomarkers with disease stage



Feature	# positive R/R Stage 3	# Total R/R Stage 3	% Prevalence R/R Stage 3	# positive de novo Stage 4	# Total de novo Stage 4	% Prevalence de novo Stage 4	p-value	q-value
TP53 (mut)	21	23	91.3	1990	2924	68.06	0.0171	0.5683
PD-L1+ (IHC)	14	23	60.87	2216	3482	63.64	0.783	0.99
TMB (High)	13	25	52	1358	3171	42.83	0.3559	0.99
KEAP1 (mut)	7	23	30.43	439	2963	14.82	0.0363	0.7051
STK11 (mut)	2	21	9.52	366	2958	12.37	0.6925	0.99
CDKN2A (mut)	1	21	4.76	324	2917	11.11	0.3556	0.99

A panel of common IO-related biomarkers are highlighted in the bar graph and table above. Increase in mutational prevalence of TP53 and KEAP1 observed in R/R were highlighted in Fig2. Differences in the other biomarkers were numerical, between the two cohorts.

Figure 5. TME estimates associated with disease stage



Feature	R/R Stage 3 (n=21)	DN Stage 4 (n=2723)	p-value	q-value
Macrophage M1	0.0583	0.0549	0.9749	0.9957
Neutrophil	0.0474	0.0531	0.5181	0.99
Macrophage M2	0.0457	0.0518	0.1436	0.9618
B cell	0.0289	0.0446	1.00E-04	0.0498
NK cell	0.0233	0.0254	0.166	0.9644
T cell regulatory (Tregs)	0.0129	0.0258	0.0037	0.3054
T cell CD8+	0.0015	0.0051	0.1687	0.9876
Monocyte	0	0	0.8986	0.99
Myeloid dendritic cell	0	0.0019	0.9103	0.99
T cell CD4+ (non-regulatory)	0	0	0.9954	0.9987

B cells (2.89 vs 4.46%, q<0.05) and Tregs (1.29 vs 2.58%; p<0.05,q>0.05) were reduced in R/R compared to DN. * q<0.05

Conclusions

- R/R patient tumors had distinct molecular alterations and immune landscapes, including an increased prevalence of biomarkers associated with immunotherapy resistance (*TP53* and *KEAP1* mutations) and reduced B- and Treg- cell fractions, which may suggest decreased likelihood of response to immunotherapy compared to patients with DN NSCLC.
- Molecular features might be able to define more aggressive interventions in the future for patients with R/R Stage III NSCLC where further treatment intensification might be needed.

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