Association of immune microenvironment to response in treatment naïve non-small cell lung cancer (NSCLC) samples with follow-up second line immunotherapy data

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background

Archival specimens collected months or years prior to starting immunotherapy are often used to identify patients for second line immune checkpoint inhibitor (ICI) treatment. PD-L1 expression and the immune microenvironment in these patients may have altered over time following multiple lines of failed standard of care (SOC) treatments.

methods

We have analysed formalin fixed paraffin embedded (FFPE) tissues in a cohort of NSCLC patients taken during resection performed as first line surgical treatment for which radiotherapy, SOC chemotherapy, and second line immunotherapy clinical follow-up data are available.

The immune microenvironment was evaluated by immunohistochemistry (IHC; n=18 patients) plus digital image analysis (CellProfiler™) for CD3 (2GV6), CD8 (SP57), CD68 (PG-M1) and CD163 (2G12). PD-L1 (22C3) was scored by a pathologist for tumour proportion score (TPS) and combined positivity score (CPS). Samples (n=22 patients) were analysed by NanoString using the PanCancer IO360™ gene expression panel. The aim of the study was to explore whether immune signatures predictive of response to ICI therapy may be identified in such samples.

results

Clinical follow-up data indicated objective response to ICI therapy for 4 patients with the mean time from initial diagnosis to ICI treatment being 2.8 years (range: 0.4 to 8.5 years). (**Table 1**). During this time these patients failed various lines of radiotherapy and SOC chemotherapy prior to receiving immunotherapy.

IHC image analysis data revealed significantly more CD3 (2.3-fold) and CD8 (2.7-fold) T cell numbers in the responder population (**Figure 1**). In addition, although CD68+ macrophage frequencies did not differ significantly between responders and non-responders, reduced M2-like CD163+ macrophage/monocyte numbers were evident for responders (**Figure 2**).

While PD-L1 expression, whether measured by IHC (data not shown), or NanoString (**Figure 3**), was found not to be significantly associated with response to immunotherapy in this small cohort of samples, gene expression analysis did identify several signatures associated significantly with response, including increased abundance of CD8 T cells, cytotoxic cells, cytotoxicity and MHC class II antigen presentation (**Figure 3**). Significant changes in the macrophage population similar to those observed by IHC were not evident by NanoString gene expression analysis.

TABLE 1. Clinical follow-up data for cases analysed by both IHC and NanoString

Gender	Age (years)	Stage at diagnosis	Sub-Type	Immunotherapy	Time from first diagnosis to immunotherapy (months)	Overall survival (OS) (in months)	Progression free survival (PFS) (in months)	Alive/Dead	Responder status
M	63	IIIB	NSCLC	Nivo	5	6	6	A	R
M	63	IIIA	ADC	Durva	16	7	7	A	R
M	57	IB	ADC	Pembro	88	No data	4	No data	R
M	71	IIA	ADC	Nivo	102	17	17	A	R
M	78	IIB	SCC	Nivo	35	5	5	D	NR
-	62	IIIA	SCC	Nivo	25	2	2	D	NR
M	59	IIIA	ADC	Nivo	12	0	0	D	NR
F	57	IIIA	SCC	Pembro	6	2	2	A	NR
M	59	IIIA	micropapillary ADC	Nivo	12	0	0	D	NR
M	70	IA	adenosquamous carcinoma	Nivo	13	7	7	D	NR
M	69	IIIA	SCC	Nivo	13	7	2	D	NR
M	62	IIIA	SCC	Nivo	18	9	9	D	NR
M	70	IB	basaloid SCC	Nivo	17	7	7	D	NR
M	78	IIB	SCC	Nivo	35	5	5	D	NR
F	67	IIB	papillary ADC	Nivo	29	5	5	A	NR
M	59	IIIB	micropapillary ADC	Pembro	48	No data	3	A	NR
M	57	IIIA	keratinizing SCC	Nivo	47	4	4	D	NR
M	64	IA-3	acinar ADC	Nivo	83	7	7	D	NR

Figure 1

Tumour Infiltrating Lymphocytes are Significantly Associated with Response

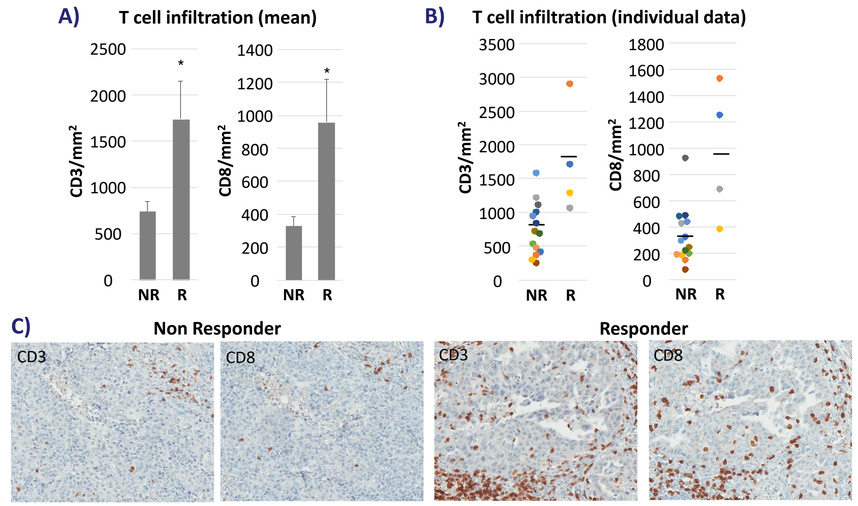


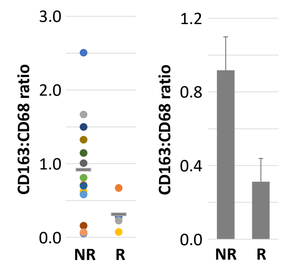
Figure 1. Analysis of tumour infiltrating lymphocytes by IHC. Samples were stained by IHC for CD3 and CD8. Digital images were acquired using an Aperio scanner and analysed using CellProfiler™ to deliver the number of immune cells/mm². **A)** Data are expressed as mean cells/mm² for non-responder (NR) versus responder (R) populations (*p<0.05), **B)** plotted as individual data. **C)** Representative images for CD3 and CD8 for NR versus R.

Figure 2

Reduced M2-Like CD163+ Macrophages/Monocytes in Responders

A) CD68+ macrophages and CD163 status

Status	CD68	CD163
Responder	1212 ± 487	538 ± 412
Non responder	987 ± 88	881 ± 172



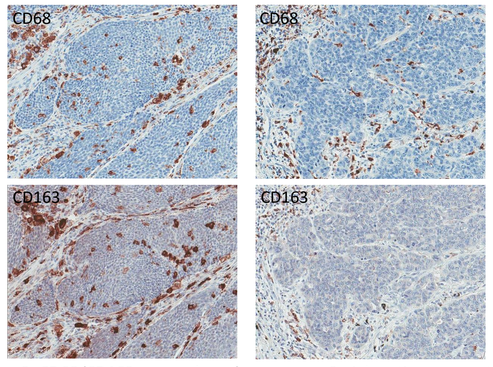


Figure 2. CD68/CD163 macrophage/monocytes. Serial sections were stained by IHC for CD68 and for CD163. Digital images were acquired using an Aperio scanner and analysed using CellProfiler™ to deliver the number of cells/mm². **A)** Mean (±SE) data for responders vs non-responders, **B)** representative images for CD68 and CD163, **C)** Individual CD163:CD68 ratios for responders vs non-responders.

Figure 3

CD8 T Cell and Cytotoxicity NanoString Signatures Associated with Response

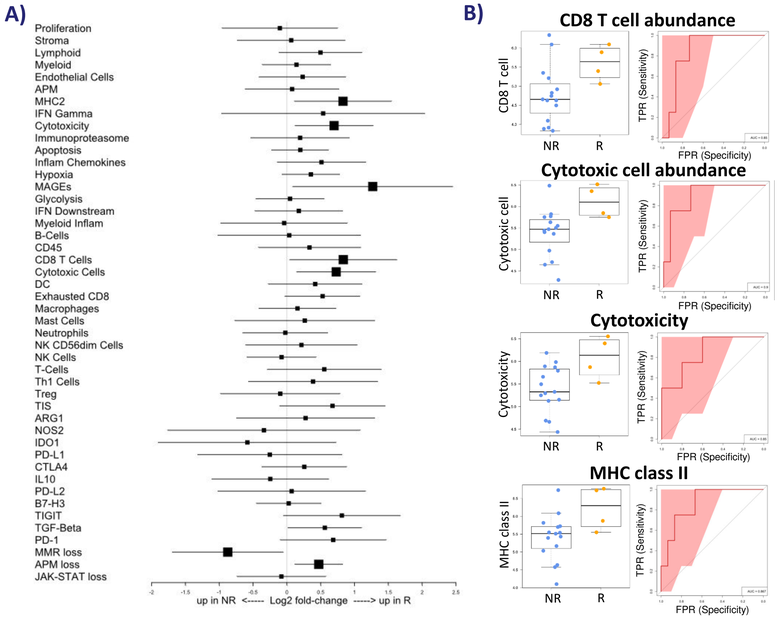


Figure 3. NanoString IO360 analysis of response. A) Forest plot of scores vs. response showing the association of signatures with response (R: responder; NR: non-responder). Points represent mean log2 fold-changes for signatures between R and NR; lines show 95% confidence intervals; larger boxes indicate statistical significance, **B)** Boxplot of scores vs. response, ● NR; ● R, and associated ROC curves illustrating the predictive performance of a signature score; predictive signatures have a curve that reaches the top left corner; shading shows 95% confidence intervals.
(Disclaimer: For Research Use Only. Not for use in diagnostic procedures.)

conclusions

- Significant increases in CD3 and CD8 T cells in the viable tumour microenvironment in responders to immunotherapy were observed by IHC. The CD8 T cell result was supported by results from the NanoString analyses.
- Response to immune checkpoint inhibitors was associated with a trend towards a switch in macrophage/monocyte phenotype.
- Taken together these data demonstrate that despite various lines of previous radiotherapy and chemotherapy spanning several years, immune profiles associated with response to second line immunotherapy can be detected in surgical first line resection samples.

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