Abstract #2666

Using “omics” to select immunotherapy and conventional therapy combinations

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Background

- Only a subset of patients respond to immunotherapies. Meaningful biomarkers are needed to predict which patients will benefit and which should be spared from potentially toxic treatment.
- Therapeutic biomarkers are expressed not only by tumor cells but also in the immune microenvironment; microenvironment-derived markers were correlated with response to checkpoint inhibitors [1].
- Current methods for evaluating PDL1 status are qualitative. Questions remain regarding choice of antibodies, thresholds for positivity, etc. [2]. Mass spectrometry (MS)-based proteomics objectively quantitates PDL1 and other therapeutically-relevant proteins with limited use of tissue samples [3,4].
- Conventional cancer therapies affect both the tumor and the relationship between tumor and immune system. Ongoing trials are evaluating combinations of immunotherapy and targeted therapy or chemotherapy.
- We hypothesized that genomic and proteomic evaluation of multiple immune biomarkers in lymphocytes and in the tumor itself would identify a signature that could (a) differentiate between responders and non-responders to checkpoint inhibitors and (b) identify candidates for combination therapy.

Methods

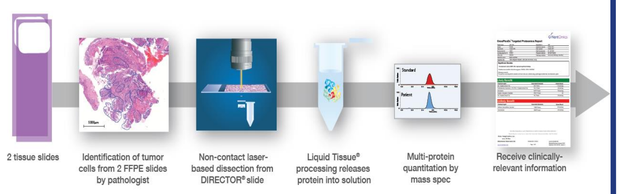
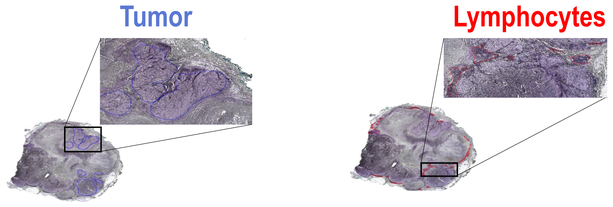


Figure 1: Dissected materials from archived tissue sections (N=2) of non-small cell lung cancer (NSCLC) and melanoma were solubilized and digested to tryptic peptides. 110 protein biomarkers, including 60 immunomarkers, were quantified in each sample using a selected reaction monitoring (SRM)- MS assay. Whole genome sequencing (WGS) and RNA-seq analyses were also performed.

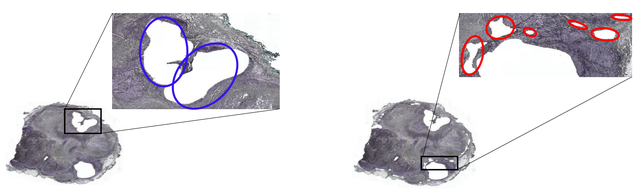
Results

Proteomic analysis of tumor and lymphocytes - Melanoma Patient

A Pre-dissection image



Post-dissection image



B

Site	Biomarkers (amol/µg)						
	PDL1	IDO1	CD3D	B7H3	B7-2	STAT1	GBP1
Tumor Only	216	313	ND	840	727	22375	15098
Tumor-Associated Lymphocytes	339	889	238	ND	538	16137	15032

Figure. 1: A. Tumor and tumor-associated lymphocytes were microdissected from melanoma tissue sections **B.** Of the 20 immuno-biomarkers that could be identified, 7 are presented.

Immunotherapy

Protein marker	Quantity (amol/µg)		Relevance to therapy selection
	Tumor	Tumor-associated lymphocytes	
PDL1 protein	216	339	Likely to respond to: atezolizumab, pembrolizumab, nivolumab
IDO1 protein	313	889	Clinical trials of IDO1 inhibitors

Sensitivity markers to immunotherapies

Marker	Status	Immunotherapy permissive
MSI status	Stable	No
Mutation rate	5.79 / Mb CDS	Yes

Chemo and targeted therapy

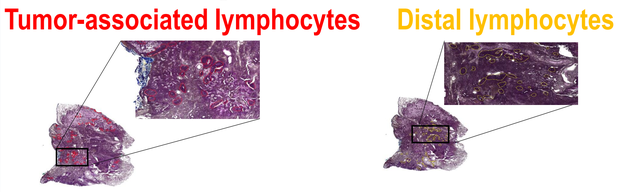
Genomic marker	Status	Relevance to therapy selection
BRAF (V600E)	Detected	Likely to respond to: vemurafenib, dabrafenib, regorafenib, sorafenib
Protein marker	**Quantity in tumor (amol/µg)**	**Relevance to therapy selection**
ERCC1	127	Resistance to platinum
TUBB3	1024	Resistance to taxane
hENT1	162	Likely to respond to gemcitabine
HER3	349	Clinical trials of HER3 inhibitors

Possible therapy selection: immunotherapy + BRAF-targeted therapy. The patient’s tumor and lymphocytes expressed markers for response to immunotherapy (PDL1 and IDO1). Molecular profiling suggested BRAF inhibitors and resistance to platinum and taxane.

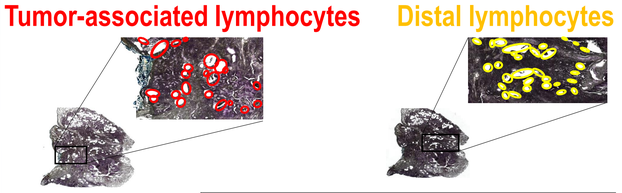
Proteomic analysis of tumor and lymphocytes - NSCLC Patient

Is the biology of tumor associated lymphocytes and distal lymphocytes similar?

Pre-dissection image



Post-dissection image



Site	Biomarkers (amol/µg)					
	PDL1	CD3D	CD3E	CD27	CD8	B7-2
Tumor Only	ND	ND	ND	ND	ND	739
Distal Lymphocytes	ND	170	927	546	803	ND
Tumor Assoicted Lymphocytes	ND	172	924	359	600	924

Figure 2. A. Tumor-associated and distal lymphocytes (>1 mm from marked tumor areas) were micro-dissected from NSCLC tissue sections and MS analysis was performed. **B.** Protein expression variation between the tumor-associated lymphocytes and distal lymphocytes were < 10% for 11 proteins and < 30% for 14 proteins.

Immunotherapy

Protein marker	Quantity (amol/µg)			Relevance to therapy selection
	Tumor	Tumor-associated lymphocytes	Distal lymphocytes	
IDO1 protein	597	521	186	Clinical trials of IDO1 inhibitors

Sensitivity markers to immunotherapies

Marker	Status	Immunotherapy permissive
MSI status	Stable	No
Mutation rate	1.18 / Mb CDS	No
STK11	Loss (0.0x)	No

Chemo and targeted therapy

Protein marker	Quantity in tumor (amol/µg)	Relevance to therapy selection
ERCC1	ND	Likely to respond to platinum
TUBB3	5765	Resistance to taxane
hENT1	79	Likely to respond to gemcitabine
TOPO1	1072	Likely to respond to irinotecan or topotecan
TOPO2A	ND	Resistance to anthracyclines
HER3	210	Clinical trials
AXL	124	Clinical trials
GPNMB	7853	Clinical trials

Possible therapy selection: platinum plus gemcitabine followed by immunotherapy: The patient’s tumor and lymphocytes expressed IDO1 protein, but genomic analysis suggested a non-inflamed tumor. Proteomic analysis suggested response to platinum and gemcitabine.

Conclusion

- Molecular profiling revealed clinically relevant immunoproteins expressed by lymphocytes, tumor or both. These may be used to select patients for approved immunotherapies or clinical trials.
- Proteomic and genomic profiling can also characterize biomarkers to inform selection of targeted therapies and chemotherapies.
- Ongoing clinical trials of immunotherapy and combination therapies could benefit from precise, quantitative molecular stratification of patients. Protein level cutoffs that correlate with response to immunotherapy are in development.
- The protein expression level of many of the immuno-biomarkers that were studied were similar between tumor-associated lymphocytes and distal lymphocytes in a single patient sample. Further analysis in large cohorts will be necessary to draw conclusions.
- Personalized medicine should include the use of proteomic as well as genomic profiling to characterize all potential biomarkers which may direct therapies.

References:
1. Erfani N, et al. Lung Cancer. 2012 Aug;77(2):306-11.
2. Velcheti V, et al. Lab Invest. 2014 Jan;94(1):107-16.
3. Nuciforo, P. et al. Mol Oncol, 2016 Jan; 10(1): 138 – 47.
4. Catenacci, DVT. et al. PLos One, 2014 Jul; 9(7): e100586.

Definition:
ND= Non Detected

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