Abstract 52 Figure 2 Patient 021 dashboard

compared patients' percentages to sample average relative abundance. Two patients, P011 shown in figure 1 and P021 shown in figure 2, are depicted using this approach.

Conclusions ATLAS can be used in real-world conditions to generate comprehensive immunological profiles of cancer patients. Individual profiles indicate that immunological constitution is heterogeneous among patients, even with the same tumor type. We propose that the addition of ATLAS to our clinical and immunological toolbox may help stratify patients to articulate truly personalized oncologic therapies.

Ethics Approval The study was approved by Loyola University Medical Center and Loyola University Chicago Ethics Board and Institutional Review Board, approval number 209364.

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PREDICTORS OF RESPONSE TO IMMUNE CHECKPOINT INHIBITOR THERAPY IN METASTATIC SOLID TUMORS: REAL WORLD EVIDENCE

Asasem Jacob*, Jianrong Wu, Jill Kolesar, Eric Durbin, Aju Mathew, Susanne Arnold, Aman Chauhan. *University of Kentucky, Lexington, KY, USA*

Background Immune checkpoint inhibitor (ICI) therapy is increasingly being used in oncology and novel predictive biomarker for efficacy and side effects are an unmet need.^{1 2} The study aims to do a comprehensive analysis of factors affecting outcome from ICI therapy with real-world data and identify potential predictive biomarkers in diverse populations. **Methods** We performed a retrospective analysis of patients with metastatic solid tumors who received ICI and underwent molecular profiling with FoundationOne® CDx panel between 2016 and 2020 at Markey Cancer Center, Lexington KY. Progression-free survival (PFS), radiological response, and autoimmune side effects were analyzed and compared with various molecular biomarkers (figure 1). Logistic regression, Fisher's exact test, Kaplan-Meier method, log-rank test, and Cox regression were used to analyze clinical features and efficacy outcomes.

Results 69 patients were included in the study (tables 1 and 2). A statistically significant improvement in PFS was observed in the PIK3 mutated cohort (median 123 vs. 23 weeks. HR=2.51. 95%CI 1.23, 5.14; table 3 and figure 2). This was independent of tumor mutational burden (TMB) status or PDL1 expression status (HR 3.24, p=0.016). PIK3 mutants had a higher overall response rate (ORR) than the wild type

(69.6% vs. 43.5%, OR 0.34; p=0.045; tables 3 and 4). PIK3 mutants had a higher risk of developing immune-related adverse events (IRAEs) (73.9% vs. 37%, p=0.004). PIK3 mutation did not associate with TMB, PDL1 expression or microsatellite stability status. Median PFS was higher in the high TMB cohort compared to the low-intermediate group and reached statistical significance (median not reached vs. 26

Abstract 53 Figure 1 Mutational analysis of patients receiving immunotherapy grouped based on radiologic response, in the order of mutational load and frequency of mutations

Abstract 53 Table 1 Baseline characteristics of the study population

Characteristics	Total (%)
Age group	
18-64	40 (58)
65-74	19 (27.5)
≥ 75	10 (14.5)
Male: Female	36 (52.2): 33 (47.8)
Race	
Caucasian	63 (91.3)
African American	4 (5.8)
Hispanic	1 (1.4)
Asian	1 (1.4)
Cancer Site	
NSCLC	26 (37.7)
SCC Head & Neck	8 (11.6)
Melanoma	7 (10.1)
Gastrointestinal	7 (10.1)
Kidney/Bladder	6 (8.6)
Small Cell lung Cancer	5 (7.2)
Hepatobiliary	2 (2.9)
Breast	2 (2.9)
Ovary and uterus	2 (2.9)
Thyroid	2 (2.9)
Merkel Cell	1 (1.4)
Unknown primary	1 (1.4)
Tobacco use	
Current/previous use	54 (78.3)
Never used	15 (21.7)
NSCLC: Non-small cell lung cancer, SCC: Squamous cell carcinoma	

Abstracts

Abstract 53 Table 2 Treatment and biomarker characteristics of study population

Characteristics	n (%)
Immunotherapy treatment	
Chemoimmunotherapy	6 (8.7)
Combination immunotherapy	10 (14.5)
Single agent immunotherapy	53 (76.8)
ICI used	
Atezolizumab	2 (2.9)
Ipilimumab-Nivolumab	11 (15.9)
Nivolumab	11 (15.9)
Pembrolizumab	45 (65.2)
IRAEs	
Any	33 (47.8)
Grade 1	7 (10.1)
Grade 2	16 (23.2)
Grade 3/4	10 (14.4)
Tumor Mutational Burden	
High	9 (13)
Intermediate	32 (46.4)
Low	28 (40.6)
PDL1 tumor proportion score	
0	14 (20.3)
1-49%	19 (27.5)
≥50	10 (14.5)
Unknown	26 (37.7)
Microsatellite stability	
Stable	62 (89.9)
Unstable	5 (7.2)
Unknown	2 (2.9)
Best Response	
CR	14 (20.3)
PR	22 (31.9)
SD	12 (17.4)
PD	21 (30.4)

Abstract 53 Figure 2 Kaplan-Meier graphs depicting progression free survival in patients based on tumor samples showing (a) High TMB and low/intermediate TMB; (b) PDL1 expression; (c) Presence of IRAEs; (d) Presence of PIK3 mutation; (e) Presence of FGFR mutation; (f) Presence of BRAF mutation

Abstract 53 Table 3 ORR based on various factors with odds ratio calculated using logistic regression model

Variable	Total	Responders (CR/PR) n (%)	Non-responders (SD/PD) n (%)	Overall response rate	OR 95% CI
All patients	69	36 (52.2)	33 (47.8)	52.2%	
Age <65	40	19 (47.5)	21 (52.5)	47.5%	0.64 (0.24 - 1.68)
Age ≥65	29	17 (58.6)	12 (41.4)	58.6%	
Male	36	16 (44.4)	20 (55.6)	44.4%	1.92 (0.74 - 5.02)
Female	33	20 (60.6)	13 (39.4)	60.6%	
Smoker	54	26 (48.1)	28 (51.9)	48.1%	2.15 (0.64 - 7.14)
Non-smoker	15	10 (66.7)	5 (33.1)	66.7%	
IRAE present	34	19 (55.9)	15 (44.1)	55.9%	0.75 (0.29 - 1.92)
IRAE absent	35	17 (48.6)	18 (51.4)	48.6%	
High TMB	9	5 (55.6)	4 (44.4)	55.6%	1.17 (0.29 - 4.78)
Low/intermediate TMB	60	31 (51.7)	29 (48.3)	51.7%	
TMB <10	46	24 (52.2)	22 (47.8)	52.2%	1.00 (0.36 - 2.72)
TMB ≥10	23	12 (52.2)	11 (47.8)	52.2%	
PDL1 0%	14	5 (35.7)	9 (64.3)	35.7%	0.45 (0.12 - 1.68)
PDL1 >1%	29	16 (55.2)	13 (44.8)	55.2%	
PIK3 mutated	23	16 (69.6)	7 (30.4)	69.6%	0.34 (0.12 - 0.97)
PIK3 wildtype	46	20 (43.5)	26 (56.5)	43.5%	
FGFR mutated	16	13 (72.2)	3 (18.8)	72.2%	0.32 (0.08 - 1.01)
FGFR wildtype	51	23 (45.1)	28 (54.9)	45.1%	
ROS1 mutated	10	9 (90.0)	1 (10.0)	90.0%	0.09 (0.01 - 0.79)
ROS1 wildtype	59	27 (45.8)	32 (54.2)	45.8%	
BRAF mutated	5	3 (60.0)	2 (40.0)	60.0%	0.71 (0.11 - 4.54)
BRAF wildtype	64	33 (51.6)	31 (48.4)	51.6%	
STK11 mutation	11	4 (36.4)	7 (63.6)	36.4%	2.15 (0.57 - 8.17)
STK11 wildtype	58	32 (55.2)	26 (44.8)	55.2%	

Abstract 53 Table 4 Identified PIK3 mutations in tumor samples, with their chromosomal position and protein changes

Tumor sample	Mutation	Type	Position	Event	Protein change	TMB	MSI
1	PIK3CB	Missense	chr3:138426129	1402G>A	E468K	high	MSS
2	PIK3C2G	CNA Amp	chr12:18435015-18800962			intermediate	MSI-L
	PIK3CA	CNA Amp	chr3:178875270-178996445			intermediate	MSI-L
3	PIK3C2G	Missense	chr12:18524203	1715C>T	A572V	high	MSS
4	PIK3CA	Missense	chr3:178922364	1133G>T	C378F	intermediate	MSS
5	PIK3C2B	Inframe	chr1:204438234	694_696delAAT	N232del	low	MSS
6	PIK3CB	Truncation	chr3:138452282	973-2A>T	splice site 973-2A>T	high	MSS
7	PIK3CA	Inframe	chr3:178916940	328_330delGAA	E110del	intermediate	MSS
8	PIK3CA	Missense	chr3:178936082	1624G>A	E542K	low	MSS
9	PIK3C2B	Missense	chr1:204438345	586G>A	E196K	intermediate	MSS
	PIK3CA	Missense	chr3:178936091	1633G>A	E545K	intermediate	MSS
10	PIK3C2B	Missense	chr1:204400879	4198A>C	T1400P	low	MSS
	PIK3C2B	Missense	chr1:204399153	4294T>C	F1432L	low	MSS
11	PIK3C2G	Missense	chr12:18793433	4130G>A	R1377H	intermediate	MSS
12	PIK3C2B	Missense	chr1:204415223	2539C>A	H847N	high	MSI-H
	PIK3CA	Missense	chr3:178916905	292T>C	F98L	high	MSI-H
13	PIK3C2B	Missense	chr1:204402506	3891G>T	K1297N	intermediate	MSS
14	PIK3C2B	Missense	chr1:204409404	3295C>T	R1099C	intermediate	MSS
15	PIK3C2G	Missense	chr12:18435626	611C>T	S204F	intermediate	MSS
16	PIK3C2B	CNA Amp	chr1:204393979-204438930			intermediate	MSS
17	PIK3CA	CNA Amp	chr3:178875270-178996445			intermediate	MSS
18	PIK3CA	Missense	chr3:178921553	1035T>A	N345K	low	MSS
19	PIK3C2B	Truncation	chr1:204438071	859_860insC	R287fs*38	high	MSI-H
	PIK3CA	Missense	chr3:178936091	1633G>A	E545K	high	MSI-H
20	PIK3C2G	Missense	chr12:18435612	597C>A	N199K	low	MSS
21	PIK3C2G	Missense	chr12:18491478	1391G>C	G464A	high	MSS
	PIK3CA	Truncation	chr3:178951886	2941C>T	Q981*	high	MSS
22	PIK3C2B	Missense	chr1:204413250	2813G>A	R938H	intermediate	MSI-L
23	PIK3C2B	Missense	chr1:204419142	2070C>G	I690M	low	MSS

Abb: CNA Amp: Copy number alteration- Amplification, MSI-H: Microsatellite Instability-High, MSI-L: MSI-Low, MSS: Microsatellite Stable.

weeks; HR=0.37. 95%CI 0.13, 1.05). PDL1 expression had no significant effect on the radiologic response, but PFS improvement in patients with tumors expressing PDL1 trended towards statistical significance (median 18 vs. 40 weeks. HR=1.43. 95%CI 0.93, 4.46). BRAF mutation conferred shorter PFS (median 17 vs. 39 weeks. HR=0.35. 95%CI 0.14, 0.91) (figure 2).

Conclusions High tumor mutational burden and PIK3 mutation conferred better progression-free survival with immunotherapy across cancer types. The improvement in PFS in PIK3 mutated patients was independent of PDL1 status or TMB. The results should prompt further evaluation of these potential biomarkers and more widespread real-world data publications to help determine biomarkers that could benefit specific populations.

Ethics Approval The study was approved by University of Kentucky Institutional Review Board, approval number 49450

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PATIENT STRATIFICATION USING CLINICAL PROTEOMICS – VALIDATED MULTIPLEXED MRM ASSAYS TO QUANTIFY HER2 AND OTHER BIOMARKERS IN CLINICAL FFPE TISSUES

Maxim Isabelle*, Michael Schirm, Gwenaél Pottiez, Rudolf Guilbaud, Lorella Di Donato. *Caprion Biosciences, Montreal, Canada*

Background The advent of precision oncology has led a shift towards biomarker-driven clinical trial designs and molecular profiling of individual patients. Identification of patients with the target biomarker profile may be useful in guiding patient selection as an enrichment strategy for clinical trials. Targeted multiple reaction monitoring mass spectrometry (MRM-MS) analysis for multiplexed quantitation of biomarker proteins in FFPE tissue provides direct, more accurate and precise quantification over current ‘gold standard’ immunohistochemistry (IHC) methods. However, MRM-MS has not yet been broadly applied to clinical trials. In this study, we demonstrate the systematic development, optimization and analytical validation of quantitative, multiplexed MRM-MS assays for robust biomarker quantification in clinical FFPE tissues, including sample analysis under GCLP. Results from an MRM panel targeting 8 clinically relevant biomarker proteins will also be shown, including the measured HER2 levels in FFPE breast tumors classified by IHC as 0, 1+, 2+ or 3+.

Methods MRM-MS biomarker panels were developed and optimized for multiplexed quantitation of ≤12 proteins, in which unique peptides derived from each target protein were monitored as a surrogate measure of protein levels. Tumor regions from FFPE tissue sections were dissected using laser capture or macrodissection, solubilized, digested with trypsin to generate peptides for analysis, spiked with fixed levels of stable isotope labeled (SIL) peptide standards, and analyzed by MRM-MS. Analytical validation was performed per NCI CPTAC guidelines, including response curves, assay repeatability, selectivity, stability, and reproducibility of endogenous detection. Clinical performance was assessed using commercially sourced FFPE-tumor tissues, including a cohort of breast tumor tissues with a wide range of HER2 expression.

Results Assay performance results were protein/peptide dependent, with sensitivity in the low pg/μg total protein range. For HER2, assay linearity was demonstrated over 2.5 to 3 orders of magnitude, with a precision and accuracy of <15% over 3 independent runs. In sample analysis, the MRM-MS was sufficiently sensitive to detect HER2 in 1 μg total protein from FFPE breast tumor classified by IHC as negative (0).

Conclusions GCLP-compliant quantitative multiplexed large-scale clinical analysis of protein biomarkers by MRM-MS in FFPE tissue is feasible and enables precise and accurate quantitation of proteins when IHC methods are unsuitable or unavailable. Data can be used for patient stratification, optimization of treatment outcomes, drug resistance

prediction, and to support clinical development of novel therapies.

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THOUSANDS OF ANTIGENS ARE RECOGNIZED IN MICE VIA ENDOGENOUS ANTIBODIES AFTER BEING CURED OF A B78 MELANOMA VIA IMMUNOTHERAPY

¹Anna Hoefges*, ¹Amy Erbe, ¹Sean McIlwain, ¹Trang Le, ¹Angie Xu, ¹Nicholas Mathers, ¹Eric Zhang, ¹Andrew Melby, ¹Claire Baniel, ¹Alexander Rakhmievich, ¹Jacquelyn Hank, ²Richard Pinapati, ²Brad Garcia, ²Jigar Patel, ¹Zachary Morris, ¹Irene Ong, ¹Paul Sondel. ¹University of Wisconsin Madison, Madison, WI, USA; ²Nimble Therapeutics, Madison, WI, USA

Background Antibodies can play an important role in innate and adaptive immune responses against cancer. Using a high-density peptide array, we assessed potential protein-targets for antibodies detected in mice cured of melanoma through a combined immunotherapy regimen. Our goal was to determine the linear peptide sequences recognized by anti-tumor antibodies produced in mice cured of melanoma following immunotherapy.

Methods Mice with GD2-expressing syngeneic B78 melanoma were treated with a combination immunotherapy (local radiation therapy + intratumoral anti-GD2 mAb linked to IL2) capable of inducing an ‘in situ vaccine’ effect (ISV), enabling mice to be cured of their tumors with long-term immune memory.¹ Naïve and immune sera were collected from these mice. Using flow cytometry, immune sera showed strong antibody-binding against B16 (parental cell line of B78 without GD2 expression). These sera were then used on a Nimble Therapeutics’ peptide-array (either whole proteome or a curated list of ~650 proteins) to determine specific antibody-binding sites, and data were analyzed using a dynamic programming method that scans adjacent peptides to determine whether a peptide is bound by antibodies. Proteins were selected if peptides were bound using immune sera but not bound with the sera from naïve or non-responding tumor-bearing mice.

Results Multiple proteins were selectively identified by immune sera that were not detected by sera from naïve or non-responding tumor-bearing mice. When focusing on the whole mouse proteome data, thousands of peptides were targeted by 2 or more mice and exhibited strong antibody binding only by immune sera. We also identified a few proteins that elicited an immune response in the naïve mouse sera that showed a significantly stronger signal in the immune sera of the same mice indicating that the cancer and/or the received therapy strengthened the immune response to these proteins.

Conclusions We are able to detect selective antibody binding to immune sera. However, we are continuing to refine our analytical methods and are further investigating the identified proteins. These peptides may potentially serve as targets for antibody-based or cellular therapies. In addition, we are examining whether some of the identified tumor-specific endogenous antibodies might be used as biomarkers to predict response to our ISV regimen and potentially other immunotherapy treatments.

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