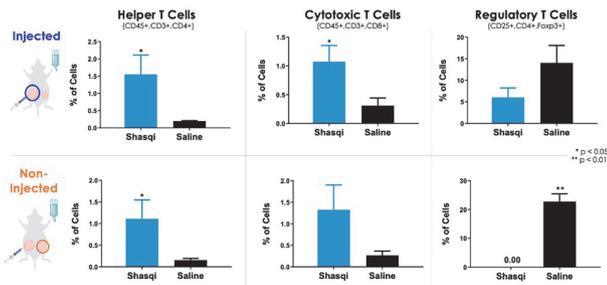


in regulatory T cells in both injected and non-injected lesions (figure 3). The T-cell response correlated with the anti-tumor efficacy data, supporting the immune activation hypothesis.



Abstract 82 Figure 3 Tumor-infiltrating immune cells in both tumors. Tumor samples were stained with antibodies and analyzed by multicolor flow cytometry. Dead cells were excluded from analysis. Results show mean ± SEM (n = 3–5 per group) as a percentage of total or marker-gated (CD4 or CD8) cells obtained from the tumor sample. Statistical significance was assessed using a corrected t test.

Conclusions SQ3370 is a proof of concept example for a novel modular approach that addresses limitations of current immuno- and cytotoxic therapies for patients with solid tumors. Local release of Dox with SQ3370 expands the therapeutic window of Dox, minimizes toxicities and leads to a robust anti-tumor response that potentially also causes immune activation against the tumors.

Acknowledgements The authors would like to thank the National Institutes of Health (NIH), the National Science Foundation (NSF), and Y Combinator.

Ethics Approval This study, project number: SQI-FFS-ON-20181119_04_v4, was approved by the Institutional Animal Care and Use Committee (IACUC) of the vendor, following the guidance of Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), accreditation number 001516.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0082>

83

THE PROGNOSTIC SIGNIFICANCE OF PERIPHERAL BLOOD BIOMARKERS IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER TREATED WITH PEMBROLIZUMAB: A CLINICAL STUDY

Kira MacDougall*, Muhammad Niazi, Jeff Hosry, Sylvester Homsy, Alexander Bershadskiy. Staten Island University Hospital, Staten Island, NY, USA

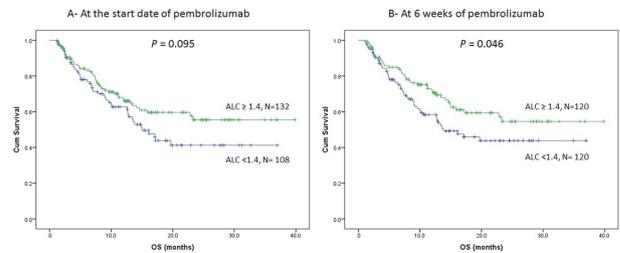
Background Pembrolizumab is an anti-programmed cell death protein 1 (PD-1) antibody used for the treatment of advanced non-small cell lung carcinoma (NSCLC). Systemic inflammation has long been associated with poor outcomes in many types of solid tumors.¹ Peripheral blood biomarkers such as absolute lymphocyte count (ALC) and absolute neutrophil count to absolute lymphocyte count ratio (ANC/ALC) serve as surrogate markers of inflammation. The aim of this study is to investigate ALC and ANC/ALC in patients with advanced NSCLC receiving pembrolizumab and determine if there is a correlation between these biomarkers and overall survival (OS).

Methods A total of 240 patients with advanced NSCLC treated with pembrolizumab at Northwell Health hospital

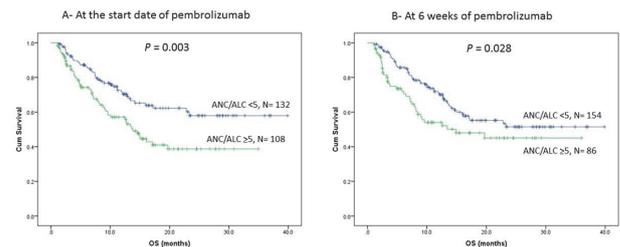
centers were included. The ALC and ANC/ALC were examined at initiation of pembrolizumab and after 6 weeks on treatment. The prognostic role of these peripheral blood biomarkers on OS were examined with Kaplan-Meier curves and a multivariable cox regression analysis.

Results Of the 240 patients, the majority were male (52%), with a median age of 67 years (interquartile range [IQR] 59–73 years), had a diagnosis of adenocarcinoma (76%), with stage IV disease (82%). PDL-1 expression was >50% in 44% of the patients. The median time on treatment with pembrolizumab was 5.7 months [IQR: 2.7–12.5]. The median ALC and ANC/ALC were significantly lower at 6 weeks of pembrolizumab compared to the start date of treatment (1.38 vs. 1.4, p<0.001) and (3.6 vs. 4.6, p<0.001) respectively. An ALC greater than 1.4 was associated with an increased OS (figure 1), at 6 weeks after initiation of pembrolizumab (p=0.046), but not at the start of treatment (p=0.095). An ANC/ALC less than 5 was associated with improved OS (figure 2), both at initiation of pembrolizumab (p=0.003) and at 6 weeks after initiation of treatment (p = 0.028). Likewise, after adjusting for potential cofounders with a multivariate analysis (table 1), a baseline ANC/ALC of 5 or higher had a significantly increased risk of death (hazards ratio (HR)=1.84; 95% confidence interval (CI), 1.21–2.79; p=0.004), compared with patients with a lower ratio.

Conclusions High ALC at time of diagnosis as well as low ANC/ALC at baseline and at 6 weeks on treatment correlated with an increased OS in patients with advanced NSCLC treated with pembrolizumab. These findings represent a readily available predictive biomarker for oncologists and may help with risk stratification and strategizing treatment plans.



Abstract 83 Figure 1 Kaplan-Meier survival estimates between groups with different ALC at the start date of pembrolizumab and at 6 weeks after initiation of pembrolizumab. There is a statistically significant difference in OS between patients with ALC < 1.4 and patients with ALC ≥ 1.4 at 6 weeks after initiation of pembrolizumab (p = 0.046), but not at the start of treatment (p = 0.095).



Abstract 83 Figure 2 Kaplan-Meier survival estimates between groups with different ANC/ALC ratio at the start date of pembrolizumab and at 6 weeks after initiation of pembrolizumab. There is a statistically significant difference in OS between patients with ANC/ALC < 5 and patients with ALC ≥ 5, both at the start date of pembrolizumab (p = 0.003) and at 6 weeks after initiation of pembrolizumab (p = 0.028).

Abstract 83 Table 1 Multivariable cox regression analysis for association of baseline peripheral blood biomarkers and overall survival.

Legend: HR, hazards ratio; CI, confidence interval; CNS, central nervous system, ANC/ALC, absolute neutrophil count to absolute lymphocyte count ratio; PDL-1, programmed death-1 ligand 1; ECOG, Eastern Cooperative Oncology Group performance scale.

| | HR | 95% CI | p-value |
|------------------------------------|------|-------------|---------|
| Age | 1.00 | 0.97 – 1.02 | 0.710 |
| CNS involvement (yes vs. no) | 1.40 | 0.85 – 2.31 | 0.190 |
| ANC/ALC > 5 (yes vs. no) | 1.84 | 1.21 – 2.79 | 0.004 |
| PDL-1 expression (> 50% vs. < 50%) | 0.54 | 0.35 – 0.83 | 0.005 |
| ECOG (3-4 vs. 1-2) | 2.58 | 2.58 – 1.67 | 0.000 |

Ethics Approval The study was approved by Zucker School of Medicine at Hofstra/Northwell at Staten Island University Hospital's IRB #: 19-0922

REFERENCE

- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;**454**(7203):436–44.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0083>

84

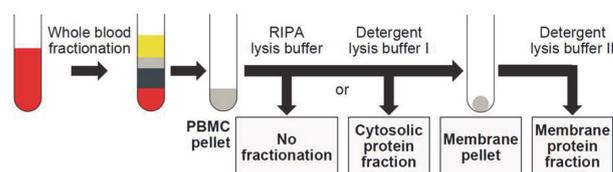
QUANTIFYING PHARMACODYNAMIC BIOMARKER CHANGES IN IMMUNO-ONCOLOGY BY MASS SPECTROMETRY

Joseph Eckenrode*, Omar Laterza, Michael Lassman. *Merck and Co., Inc., Kenilworth, NJ, USA*

Background Quantifying pharmacodynamic biomarker changes enables decision making and clinical trials in drug development. Pharmacodynamic biomarkers are used to determine the effects of treatment on disease. Mass spectrometry offers a quantitative, selective, and multiplex platform for pharmacodynamic protein biomarker analysis in clinical samples (e.g. blood and tumor) that is feasible across multiple sample conditions (e.g. fresh, frozen and formalin-fixed paraffin-embedded (FFPE)). To date, however, methodologies for targeted protein analysis by mass spectrometry (i.e. quantitative proteomics) are underdeveloped for application in immunology.

Methods To address this, we sought to extract the immunology-associated T cell membrane proteins CD3, CD4 and CD8 from peripheral blood mononucleate cells (PBMC) and develop a multiplexed mass spectrometry method to quantify their expression. PBMC were isolated from whole blood and using detergent-based lysis buffers fractionated into a cytosolic and membrane protein lysate (figure 1). Analytical methods were then developed to detect proteotypic peptides of all three proteins (table 1 and figure 6) from the lysates by mass spectrometry.

Results CD3, CD4 and CD8 were detected in the membrane protein fraction but not in the cytosolic protein fraction after whole-proteome tryptic digestion using a filter-aided sample preparation (or FASP) technique but with a signal-to-noise ratio of ≤ 2.0 (figure 2). Applying an additional immunoaffinity (IA) enrichment step with antibody-conjugated magnetic beads, prior to digestion, dramatically improved the analyte signal-to-noise ratios to > 100 (figure 3). Reverse-phase nano-flow liquid chromatography (LC) was used to separate all three analytes in multiplex over a 12-minute run prior to tandem mass analysis (MS/MS) (figure 4). Together, this IA-LC-

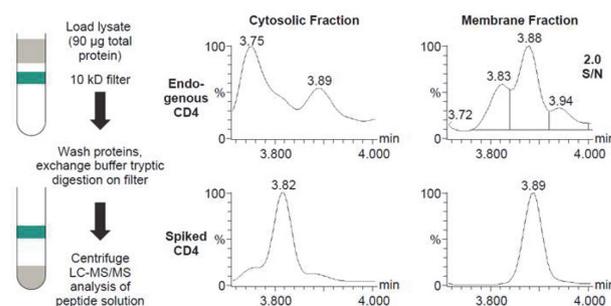


Abstract 84 Figure 1 Detergent-based protein extraction and fractionation of PBMC

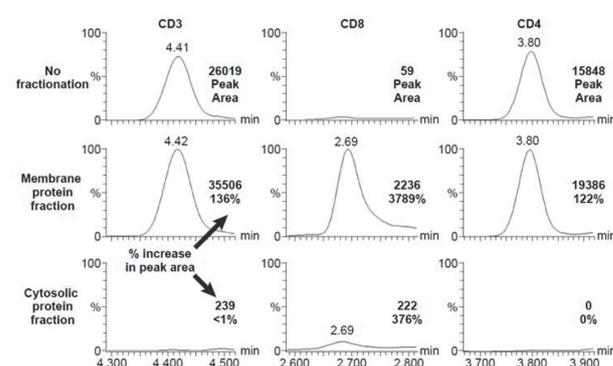
Abstract 84 Table 1 Surrogate peptides for selective protein analysis by MS/MS after tryptic digestion

| Protein | CD3 | CD8 | CD4 |
|----------------------|----------------|---------------|---------------|
| Tryptic peptide | GSKPEDANFYLRLR | AAEGLDTQR | ILGNQGSFLTK |
| MRM transition (m/z) | 558.2 > 799.3 | 480.7 > 818.4 | 589.3 > 951.4 |

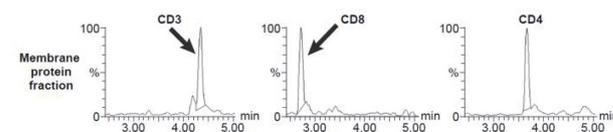
MS/MS method resulted in detection of endogenous CD3, CD4 and CD8 proteins from small volumes of whole blood (< 0.1 mL) and the analyte responses were linear over at least two orders of magnitude (figure 5).



Abstract 84 Figure 2 Filter-aided sample preparation (FASP) for whole-proteome analysis



Abstract 84 Figure 3 Immunoaffinity enrichment of proteins from PBMC lysates



Abstract 84 Figure 4 Representative multiplex analysis from 1mL of whole blood