Methods 30 pts with stage III B/C/D MEL were enrolled. Pre-operatively, CMP-001 was dosed at 5 mg subcutaneous (SC, 1st), then 10 mg IT (2nd-7th) weekly; Nivo was dosed 240 mg q2 weeks for 3 doses – both agents given for 7 weeks. Post-operatively, Nivo was dosed 480 mg q4 weeks with CMP-001 5 mg q4 weeks SC for 48 weeks. Primary end-points included major pathologic response rate (MPR), and incidence of dose-limiting toxicities (DLT). Secondary end-points were radiographic response, relapse-free survival (RFS) and overall survival (OS). Pathological response was scored blinded by pathologists based on residual volume of tumor (RVT) using prior specified cutoffs:[4] [6]0% (complete response, pCR); 0%<rvt<rvt50% (non-response, pNR). Radiographic response was assessed using RECIST v1.1. Sequential blood draws and tumor biopsies were collected and analyzed for CD8+ T cell infiltrate (TIL), multiparameter flow cytometry (MFC) and multiplex immunofluorescence (mIF).

Results 30 pts with regionally advanced MEL were enrolled, of stages IIIB (57%), IIIC (37%), IIID (7%). 29/30 (97%) of pts completed 7 weeks of neoadjuvant Nivo/CMP; while 1 pt had a delay in surgery related to a pre-operative infection unrelated to therapy. No DLTs were reported; grade 3/4 irAE were reported in 3 pts (11%) leading to CMP-001 discontinuation in 2 pts (7%). Radiographic responses were seen in 13 pts (43%), while 9 pts (30%) had stable disease and 8 pts (27%) had progressive disease. Pathological responses (RVT <50%) were seen in 70% of pts: pCR 15 (50%), pMR 3 (10%), 3 pPR (10%); only 9 (30%) had pNR. Pathological responders (pCR/pMR) had increased CD8+ TIL and CD303+ pDC intra-tumorally by mIF; and peripherally activated PD1+/Ki67+ CD8+ T cells by MFC.

Conclusions Neoadjuvant CMP/Nivo has acceptable toxicity and promising efficacy. MPR is 60% in 30 pts. 1-year RFS was 82% (all pts) and 89% (among those with pCR/pMR); median RFS is 9 months (among pNR/pPR) and not reached (among pCR/pMR). Response is associated with evidence of immune activation intra-tumorally and peripherally. IT CMP001 increases clinical efficacy of PD-1 blockade with minimal additional toxicity in pts with regionally advanced MEL. Further study of this combination in high-risk resectable MEL is planned.

Trial Registration Clinical trial information: NCT03618641

Ethics Approval The study was approved by University of Pittsburgh's Institutional Review Board, approval number MOD19040237-002.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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304 | **INTRATUMORAL INJECTION OF CMP-001, A TOLL-LIKE RECEPTOR 9 (TLR9) AGONIST, IN COMBINATION WITH PEMBROLIZUMAB REVERSED PROGRAMMED DEATH RECEPTOR 1 (PD-1) BLOCKADE RESISTANCE IN ADVANCED MELANOMA**

[1]Mohammed Milhem*, [1]Yousef Zakharia, [2]Diwakar Davar, [3]Elizabeth Buchbinder, [4]Theresa Medina, [5]Adil Daud, [6]Antoni Ribas, [7]Jiaxin Niu, [8]Geoffrey Gibney, [9]Kim Margolin, [10]Anthony Olszanski, [11]Inderjit Mehmi, [12]Takami Sato, [13]Montaser Shaheen, [14]Aaron Morris, [15]David Mauro, [6]Katie Campbell, [14]Heather Kelley, [2]Riyue Bao, [1]George Weiner, [2]Jason Luke, [14]Arthur Krieg, [14]James Wooldridge, [2]John Kirkwood. [1]University of Iowa, Iowa City, IA, USA; [2]University of Pittsburgh Medical Center, Pittsburgh, PA, USA; [3]Dana-Farber Cancer Institute, Boston, MA, USA; [4]University of Colorado Denver, Aurora, CO, USA; [5]University of California San Francisco, San Francisco, CA, USA; [6]University of California Los Angeles, Los Angeles, CA, USA; [7]Banner MD Anderson Cancer Center, Gilbert, AZ, USA; [8]Georgetown Lombardi Comprehensive Cancer, Washington, DC, USA; [9]City of Hope, Duarte, CA, USA; [10]Fox Chase Cancer Center, Philadelphia, PA, USA; [11]The Angeles Clinic and Research Institut, Los Angeles, CA, USA; [12]Thomas Jefferson University, Philadelphia, PA, USA; [13]University of Arizona, Tucson, AZ, USA; [14]Checkmate Pharmaceuticals Inc., Cambridge, MA, USA; [15]Prelude Therapeutics Inc., Wilmington, DE, USA

Background Therapeutic options are limited for patients with advanced melanoma that is refractory to PD-1 blockade. This study was performed in this patient population to assess the safety and antitumor activity of CMP-001, a CpG-A TLR9 agonist packaged within a virus-like particle.

Methods Patients were eligible for this 2-part, open-label, multicenter, phase 1b study if they had metastatic/unresectable melanoma and stable disease after =12 weeks or progressive disease (PD) on/after anti-PD-1 therapy. Part 1 evaluated CMP-001 plus pembrolizumab dose-escalation and dose-expansion. Part 2 evaluated CMP-001 monotherapy. Accessible lesion(s) were injected intratumorally with CMP-001, at a polysorbate 20 (PS20) concentration of either 0.01% or 0.00167%. The Part 1 primary objective was to identify the recommended phase 2 dose (RP2D) and schedule of CMP-001 plus pembrolizumab, while the Part 2 primary objective was to assess the safety of CMP-001 monotherapy. Secondary objectives for both parts were a preliminary assessment of antitumor activity of CMP-001 plus pembrolizumab and CMP-001 monotherapy, and the overall safety profile and pharmacodynamics of the combination.

Results In Part 1 (N=159) and Part 2 (N=40), 93.1% and 80.0% of patients had PD as their last response to prior anti–PD-1 therapy, respectively. The most common treatment-related adverse events (TRAEs; >25%) were flu-like symptoms (Parts 1 and 2) and injection-site reactions (Part 1). Grade 3/4 TRAEs were reported in 36.5% (Part 1) and 22.5% (Part 2) of patients, the most common being hypotension (Part 1:

Abstract 304 Table 1 Best ORR With CMP-001 Plus Pembrolizumab and CMP-001 Monotherapy

	Part 1: CMP-001 + Pembrolizumab Escalation and Expansion			Part 2: Monotherapy
	Intent-to-Treat Population (N=159)	CMP-001 PS20 0.01% + pembrolizumab (n=98)	CMP-001 PS20 0.00167% + pembrolizumab (n=61)	CMP-001[a] (N=40)
Best ORR (RECIST v1.1), % (95% CI)	18.9[b] (13.1, 25.9)	23.5 (15.5, 33.1)	11.5 (4.8, 22.3)	17.5[c] (7.3, 32.8)
Best ORR (including post-PD responders), % (95% CI)	23.3 (17.0, 30.7)	27.6 (19.0, 37.6)	16.4 (8.2, 28.1)	NA
Best response, n				
CR	8	7	1	0
PR	22	16	6	7
SD	35	17	19	13
PD	74	46	27	20
Post-PD response	7	4	3	–
Not evaluable[d]	13	8	5	–

CR, complete response; NA, not applicable; ORR, objective response rate; PD, progressive disease; PR, partial response; PS20, polysorbate 20; RECISTv1.1, Response Evaluation Criteria In Solid Tumors version 1.1; SD, stable disease.
Patients were dosed at CMP-001 starting doses of 1, 3, 5, 7.5, or 10 mg, using one of 2 dosing schedules. In Schedule A, CMP-001 was administered once weekly for 7 weeks, then once every 3 weeks until the patient discontinued treatment. In Schedule B, CMP-001 was administered once weekly for 2 weeks, then once every 3 weeks until the patient discontinued treatment. [a]Includes CMP-001 PS20 0.01% and PS20 0.00167%.
[b]Includes 26 confirmed responders. [c]Includes 4 confirmed responders. [d]Discontinued prior to having a follow-up scan.
Data cutoff: June 1, 2020.

6.9%; Part 2: 5.0%). No Grade 5 TRAEs were observed. In Part 1, the best objective response rate (ORR; RECIST v1.1) in patients treated with pembrolizumab and CMP-001 (PS20 0.01%) was 23.5% (23/98), while CMP-001 PS20 (0.00167%) resulted in a lower ORR of 11.5% (7/61). Seven additional patients had a delayed response after initial PD (table 1). The median duration of response was >1 year. In the 37 RECIST v1.1 and post-progression responders, the mean regression in injected and noninjected target lesions was 54.7% and 52.7%, respectively. In Part 2, the best ORR with CMP-001 monotherapy was 17.5% (7/40 patients); the response duration was shorter than in Part 1. Intratumoral CMP-001 PS20 0.01% 10 mg was selected as the RP2D.

Conclusions Intratumoral CMP-001 was well-tolerated and provided both local and distant responses in patients with advanced melanoma with disease progression on prior PD-1 blockade. CMP-001 monotherapy induced systemic tumor regression in some patients, but duration of response was substantially increased by the addition of pembrolizumab.

Trial Registration NCT02680184
Ethics Approval This study was approved by the WCG-WIRB, WIRB approval tracking number 20152597.
Consent N/A

REFERENCES
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305 **TECHNICAL CONSIDERATIONS FOR NORMALIZING DIGITAL SPATIAL PROFILING DATA WITH MULTIPLE WITHIN-PATIENT SAMPLES**

[1]Tyler Hether, [2]Tim Howes*, [2]Christine Spencer, [3]Travis Hollman, [1]Jason Reeves, [2]Danny Wells, [4]Claire Friedman, [2]Theresa LaVallee, [3]Jedd Wolchok, [1]Sarah Warren. [1]NanoString Technologies, Inc., seattle, WA, USA; [2]Parker Institute for Cancer Immunotherapy, San Francisco, CA, USA; [3]Memorial Sloan Kettering Cancer Center, Parker Institute for Cancer Immunotherapy, New York, NY, USA; [4]Memorial Sloan Kettering Cancer Center, Weill-Cornell Medical College, New York, NY, USA

Background NanoString's GeoMx Digital Spatial Profiling (DSP) technology enables profiling of gene or protein expression from fresh or archival tissues. Specific regions of interest (ROIs) are identified via fluorescently labeled visualization markers. Within a given ROI, oligonucleotide tags from labeled, incubated antibodies can be released by area of interest (AOI)-specific exposure to UV light. With DSP, multiple AOIs can be collected within an individual tissue and/or within an individual patient. As with other technologies, technical variation that needs to be accounted before meaningful conclusions can be drawn.[1] Herein, we discuss technical considerations for normalizing and examining DSP data with multiple within-sample observations. We have two goals: 1) determine how different technical artifacts affect raw protein or RNA counts 2) provide guidelines for normalization strategies based on the biological questions of interest. To address these, we examine a recent melanoma dataset to quantify protein expression levels in tumor and stroma AOIs and to determine associations of specific proteins with clinical benefit (CB) from immunotherapy.

Methods Seventy-nine segmented ROIs containing matched tumor and stroma compartments were examined from eight patients at baseline (range: 4–12 ROIs). Five of these patients showed CB, defined as complete response, partial response, or remaining progression-free for 6 months. Following UV cleavage, liberated oligonucleotide tags were collected via microcapillary into a microtiter plate, and then processed using the nCounter Prep Station and Digital Analyzer as per manufacturer instructions.

Results Each AOI included 57 protein counts and six categories of control molecules/metrics (e.g., isotype molecules, AOI-specific cellularity). Before normalization, we examined controls and excluded those showing correlations with CB or segmentation type. We compared different normalization strategies including area and isotype normalization, upper quartile, and RUV.[2] For each strategy, we used linear and negative binomial mixed models to correlate protein expression with CB status, segmentation type, or their interaction. Findings consistent throughout many analysis combinations included higher MART1 expression in the CB group, lower PD-L1 and Ki-67 in the CB group, and lower HLA-DR expression in tumor segments of the CB group.

Conclusions ROIs can vary in size, cellularity, and staining, and normalization is important to account for technical differences when quantifying expression in spatial profiling studies. Normalization choices can affect outcome, and it is important to check whether proposed control proteins are in fact unassociated with the biological factors of interest. Mixed modeling approaches can be used to simultaneously model variation between ROIs within a sample and determine differences between sample groups.

Trial Registration ClinicalTrials. gov NCT02731729
Ethics Approval The study protocol and amendments were approved by the IRB of each participating institute. Written informed consent was obtained from all patients before conducting any study-related procedures.

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