Skin proteomic analysis of immune activation associated with regression of melanoma metastases induced by diphencyprone

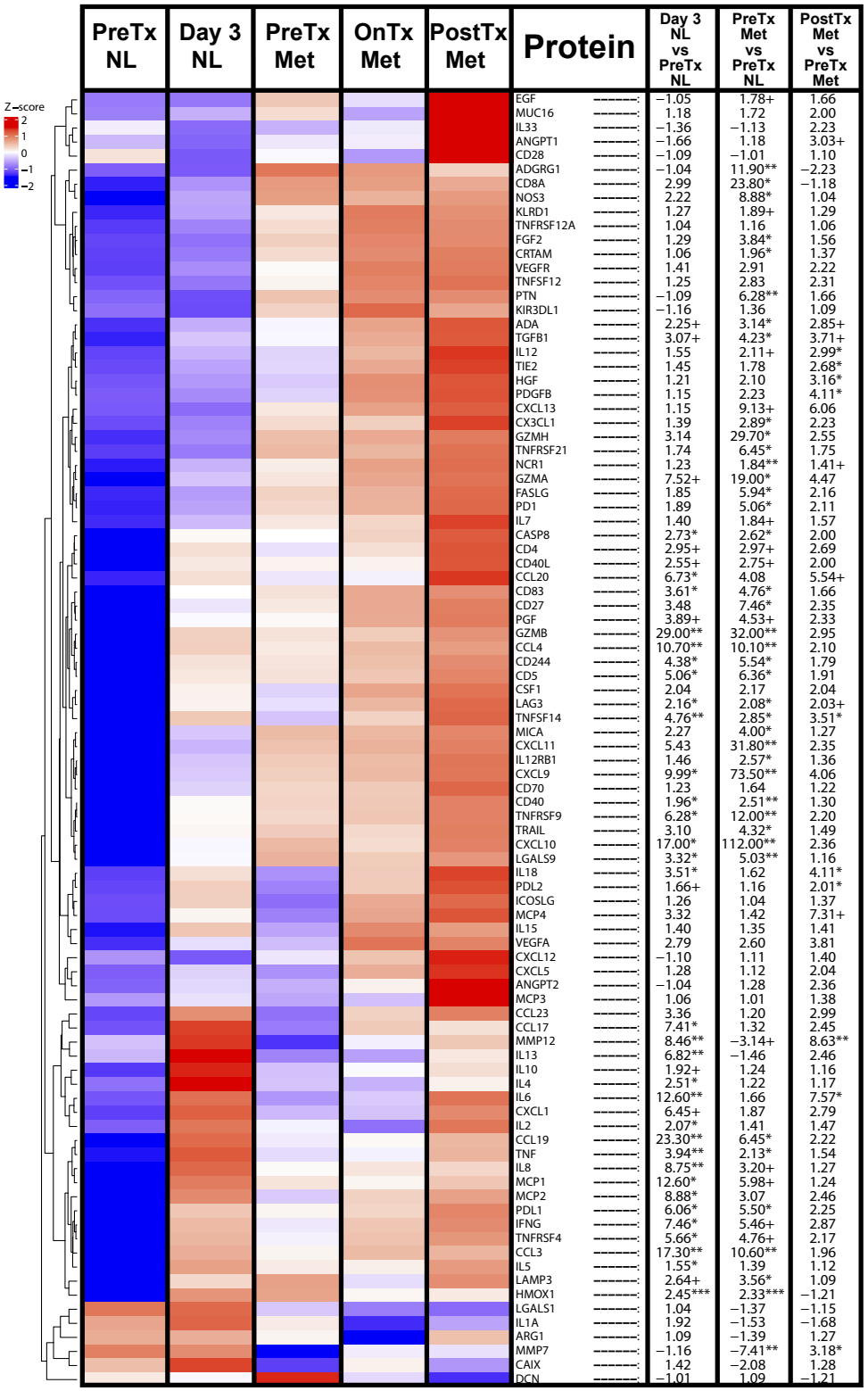
To the Editor: Diphencyprone (DPCP), a hapten that causes delayed-type hypersensitivity reactions, has been used to treat warts, alopecia areata, and cutaneous metastases in melanoma patients.[1] Previously, our group performed transcriptomic analysis of skin biopsies from melanoma metastases treated with topical DPCP, revealing significant increases in Th1-related genes but not in genes of other major T-cell subsets.[2] While gene expression profiling has long been used as a proxy for protein expression, correlation between messenger RNA (mRNA) and protein levels is inconsistent due to factors such as post-transcriptional regulation. Therefore, to more precisely ascertain the mechanisms involved in immune-mediated tumor regression induced by DPCP, we performed proteomic analysis of skin biopsies from 5 patients with cutaneous melanoma metastases treated with DPCP twice weekly for 7 to 14 weeks (Supplemental Material and Methods and Supplementary Table I, available via Mendeley at https://doi.org/10.17632/f5ttw4mygy.1). In this study, we assessed 96 proteins using the Olink immuno-oncology panel for the 5 patients who underwent molecular profiling.

All patients had expected skin inflammation and at least partial regression of skin metastases, without systemic side effects. There were 10 proteins (interleukin 6 [IL-6], IL-12, IL-18, TIE2, HGF, PDGFB, TNFSF14, PD-L2, MMP7, MMP12) significantly upregulated ($P < .05$) in post-treatment versus pretreatment metastases (Fig 1), including biomarkers associated with Th1 response (IL-12), innate immunity (IL-6), and immune checkpoints (PD-L2) ($P < .05$). All of these proteins had progressively increased expression in cutaneous metastases with repeated DPCP applications, and similar increases were observed in nonlesional skin of these patients following a single DPCP application. Prior work has shown that administration of IL-18 in mice increases activated T cells and natural killer cells that contribute to effective antimelanoma immunity,[3] so the significant upregulation of this protein in our study may contribute to the successful skin metastasis regression observed in our patients. TNFSF14 also likely plays an active role in immune-mediated regression, as *in vivo* studies have demonstrated an association between the expression of this protein in melanoma metastasis lesions and proliferation of T cells.[4] Additionally, there were 40 proteins significantly upregulated in pretreatment metastases versus untreated nonlesional skin ($P < .05$) (Fig 1), suggesting baseline immune activation in melanoma metastases. Upon DPCP treatment, changes in nonlesional skin positively correlated with those in skin metastases (Supplementary Fig 1, available via Mendeley at https://doi.org/10.17632/f5ttw4mygy.1; Spearman correlation = 0.61, $P < .0001$).

We performed a correlation analysis between the proteomic data and the previously published mRNA data,[2] which both were completed on the same tissue samples, to assess the relationship between these 2 "omic" approaches (Fig 2). Correlation was poor ($r = -0.1$, $P = .4012$), including for proteins that were significantly upregulated by proteomics (IL-6 and TNFSF14) (both $P < .05$). Gene expression from mRNA is variably correlated with protein expression, the latter being influenced by many regulatory processes prior to translation. Another explanation for the discrepancy observed between mRNA and protein expression includes the shorter half-life of mRNA molecules compared to proteins.[5] Despite our limited sample size, we were able to observe statistically significant protein expression changes, and this study is, to our knowledge, the first to assess the proteomic signature of successful immune-mediated regression of cutaneous melanoma metastases induced by a topical immunomodulator. Given the lack of correlation between mRNA and protein expression, along with the relatively greater feasibility of measuring protein compared to gene expression levels, our study demonstrates that proteomic analysis can give unique and potentially complementary insights into immune reactions induced by topical immunotherapy.

Joseph Han, BS,[a] Joel Correa da Rosa, PhD,[a] Shayan Owji, BS,[a] Daniel Yassky, BSE,[a] Yen Luu, BA,[b] Yeriel Estrada, BS,[a] Jonathan Ungar, MD,[a] Andrew Ji, MD,[a] James G. Krueger, MD, PhD,[c] and Nicholas Gulati, MD, PhD[a]

From the Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York[a]; School of Medicine, University of Missouri-Kansas City, Kansas City, Missouri[b]; and Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York.[c]



Protein	Day 3 NL vs PreTx NL	PreTx Met vs PreTx NL	PostTx Met vs PreTx Met
EGF	−1.05	1.78+	1.66
MUC16	1.18	1.72	2.00
IL33	−1.36	−1.13	2.23
ANGPT1	−1.66	1.18	3.03+
CD28	−1.09	−1.01	1.10
ADGRG1	−1.04	11.90**	−2.23
CD8A	2.99	23.80*	−1.18
NOS3	2.22	8.88*	1.04
KLRD1	1.27	1.89+	1.29
TNFRSF12A	1.04	1.16	1.06
FGF2	1.29	3.84*	1.56
CRTAM	1.06	1.96*	1.37
VEGFR	1.41	2.91	2.22
TNFSF12	1.25	2.83	2.31
PTN	−1.09	6.28**	1.66
KIR3DL1	−1.16	1.36	1.09
ADA	2.25+	3.14*	2.85+
TGFB1	3.07+	4.23*	3.71+
IL12	1.55	2.11+	2.99*
TIE2	1.45	1.78	2.68*
HGF	1.21	2.10	3.16*
PDGFB	1.15	2.23	4.11*
CXCL13	1.15	9.13+	6.06
CX3CL1	1.39	2.89*	2.23
GZMH	3.14	29.70*	2.55
TNFRSF21	1.74	6.45*	1.75
NCR1	1.23	1.84**	1.41+
GZMA	7.52+	19.00*	4.47
FASLG	1.85	5.94*	2.16
PD1	1.89	5.06*	2.11
IL7	1.40	1.84+	1.57
CASP8	2.73*	2.62*	2.00
CD4	2.95+	2.97+	2.69
CD40L	2.55+	2.75+	2.00
CCL20	6.73*	4.08	5.54+
CD83	3.61*	4.76*	1.66
CD27	3.48	7.46*	2.35
PGF	3.89+	4.53+	2.33
GZMB	29.00**	32.00**	2.95
CCL4	10.70**	10.10**	2.10
CD244	4.38*	5.54*	1.79
CD5	5.06*	6.36*	1.91
CSF1	2.04	2.17	2.04
LAG3	2.16*	2.08*	2.03+
TNFSF14	4.76**	2.85*	3.51*
MICA	2.27	4.00*	1.27
CXCL11	5.43	31.80**	2.35
IL12RB1	1.46	2.57*	1.36
CXCL9	9.99*	73.50**	4.06
CD70	1.23	1.64	1.22
CD40	1.96*	2.51**	1.30
TNFRSF9	6.28*	12.00**	2.20
TRAIL	3.10	4.32*	1.49
CXCL10	17.00*	112.00**	2.36
LGALS9	3.32*	5.03**	1.16
IL18	3.51*	1.62	4.11*
PDL2	1.66+	1.16	2.01*
ICOSLG	1.26	1.04	1.37
MCP4	3.32	1.42	7.31+
IL15	1.40	1.35	1.41
VEGFA	2.79	2.60	3.81
CXCL12	−1.10	1.11	1.40
CXCL5	1.28	1.12	2.04
ANGPT2	−1.04	1.28	2.36
MCP3	1.06	1.01	1.38
CCL23	3.36	1.20	2.99
CCL17	7.41*	1.32	2.45
MMP12	8.46**	−3.14+	8.63**
IL13	6.82**	−1.46	2.46
IL10	1.92+	1.24	1.16
IL4	2.51*	1.22	1.17
IL6	12.60**	1.66	7.57*
CXCL1	6.45+	1.87	2.79
IL2	2.07*	1.41	1.47
CCL19	23.30**	6.45*	2.22
TNF	3.94**	2.13*	1.54
IL8	8.75**	3.20+	1.27
MCP1	12.60*	5.98+	1.24
MCP2	8.88*	3.07	2.46
PDL1	6.06*	5.50*	2.25
IFNG	7.46*	5.46+	2.87
TNFRSF4	5.66*	4.76+	2.17
CCL3	17.30**	10.60**	1.96
IL5	1.55*	1.39	1.12
LAMP3	2.64+	3.56*	1.09
HMOX1	2.45***	2.33***	−1.21
LGALS1	1.04	−1.37	−1.15
IL1A	1.92	−1.53	−1.68
ARG1	1.09	−1.39	1.27
MMP7	−1.16	−7.41**	3.18*
CAIX	1.42	−2.08	1.28
DCN	−1.01	1.09	−1.21

Fig 1. Heatmap of immuno-oncology proteins in melanoma metastases before, during, and after DPCP treatment in addition to nonlesional skin before and after DPCP application, with biomarkers grouped by unsupervised hierarchical clustering. *Red boxes* correspond to upregulated proteins while *blue boxes* correspond to downregulated protein expression values. The corresponding table lists markers and respective fold changes in skin biopsies of PreTx NL, day 3 NL, PreTx Met, OnTx Met, and PostTx Met, compared to each other. $+P < .1$.

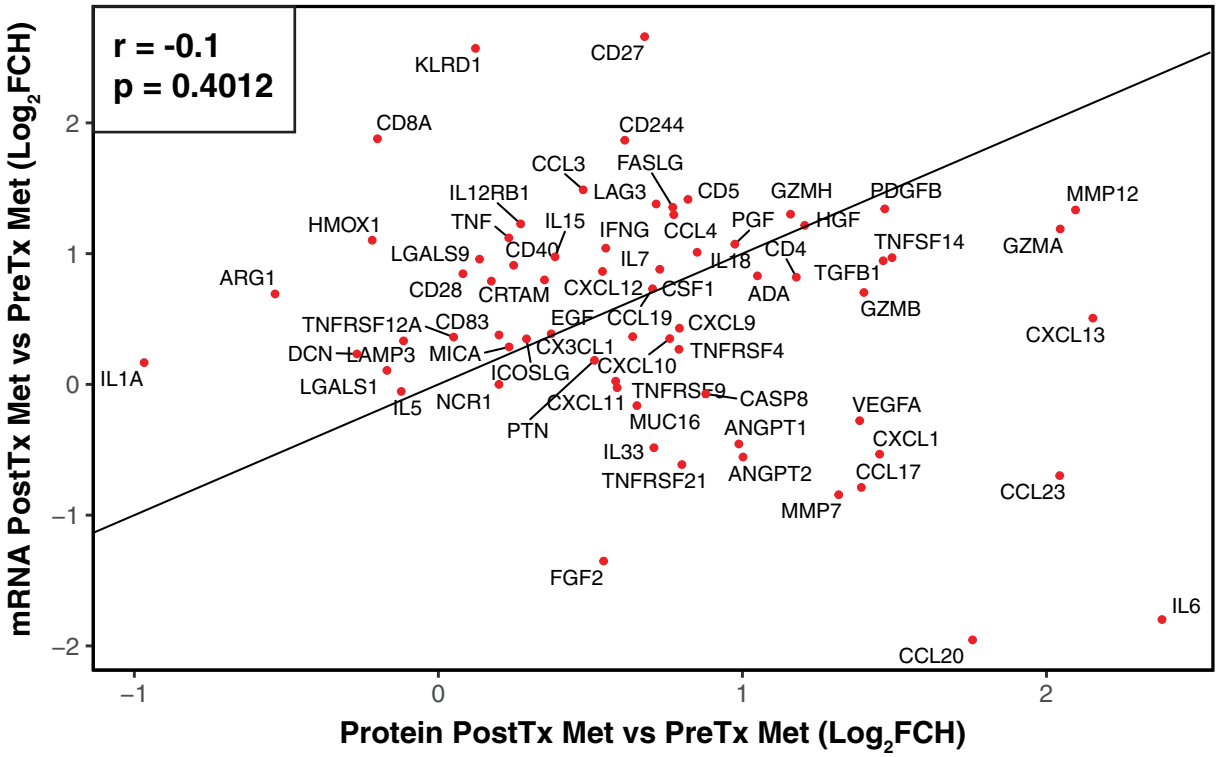


Fig 2. Scatterplot depicting the log2 FCH of mRNA microarray (y-axis) versus the log2 FCH of Olink proteomics (x-axis) of melanoma metastasis lesions before and after DPCP treatment from the same skin samples. Spearman correlation and its significance was shown in the upper left-hand corner (r = −0.1, *P* = .4012). *DPCP*, Diphencyprone; *FCH*, fold change; *PreTx Met*, melanoma metastasis before DPCP treatment; *PostTx Met*, melanoma metastasis after DPCP treatment; *r*, Spearman correlation.

Funding sources: None.

IRB approval status: Reviewed and approved by The Rockefeller University IRB; approval #JKR-0788.

Statement of prior presentation: Late-Breaking Oral Presentation at the 2022 American Academy of Dermatology (AAD) Annual Meeting.

Key words: abscopal; biomarker; clinical trial; cutaneous; diphencyprone; diphenylcyclopropenone; DPCP; hapten; immune activation; immune checkpoint; immunotherapy; inflammation; interleukin; melanoma; metastases, mRNA; Olink; PDL2; proteomic; T helper 1; Th1; topical; tumor regression.

Correspondence to: Nicholas Gulati, MD, PhD, Department of Dermatology, Icahn School of Medicine at Mount Sinai, 5 East 98th St, Fifth Floor, New York, NY 10029

E-mail: nicholas.gulati@mssm.edu

Conflicts of interest

None disclosed.

REFERENCES

1. Damian DL, Saw RP, Thompson JF. Topical immunotherapy with diphencyprone for in transit and cutaneously metastatic melanoma. *J Surg Oncol*. 2014;109(4):308-313. https://doi.org/10.1002/jso.23506
2. Gulati N, Garcet S, Fuentes-Duculan J, et al. Molecular profiling of immune activation associated with regression of melanoma metastases induced by diphencyprone. *J Invest Dermatol*. 2016;136(10):2101-2103. https://doi.org/10.1016/j.jid.20 16.06.611
3. Zhou T, Damsky W, Weizman OE, et al. IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy.

*P < .05. **P < .01. ***P < .001. *NL*, Nonlesional skin after single DPCP application; *DPCP*, diphencyprone; *OnTx Met*, melanoma metastasis during DPCP treatment; *PreTx Met*, melanoma metastasis before DPCP treatment; *PreTx NL*, nonlesional skin before DPCP application; *PostTx Met*, melanoma metastasis after DPCP treatment.

Nature. 2020;583(7817):609-614. https://doi.org/10.1038/s41586-020-2422-6

4. Mortarini R, Scarito A, Nonaka D, et al. Constitutive expression and costimulatory function of LIGHT/TNFSF14 on human melanoma cells and melanoma-derived microvesicles. *Cancer Res*. 15 2005; 65(8):3428-3436. https://doi.org/10.1158/0008-5472.CAN-04-3239
5. Mauger DM, Cabral BJ, Presnyak V, et al. mRNA structure regulates protein expression through changes in functional half-life. *Proc Natl Acad Sci U S A*. 2019;116(48):24075-24083. https://doi.org/10.1073/pnas.1908052116

https://doi.org/10.1016/j.jdin.2022.08.006