Bespoke circulating tumor DNA as a biomarker for treatment response in a refractory Merkel cell carcinoma patient

John Yeakel, MD,[a] Aarthi Kannan, BA,[b] Nicole Hook Rattigan, PhD,[c] Maki Yamamoto, MD,[d] Alexey Aleshin, MD, MBA,[c] Jeremy P. Harris, MPhil, MD,[a] and Ling Gao, MD, PhD[b,e,f]
Irvine and Long Beach, California, and Austin, Texas

Key words: circulating tumor DNA; hypofractionated radiation; Merkel cell carcinoma; T-VEC.

INTRODUCTION

Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer with climbing incidence and a dismal 5-year survival rate ≤18%.[1] While immune checkpoint inhibitors (ICIs) have become standard of care for advanced MCC,[2] there is no effective alternative for patients ineligible or resistant to immunotherapy. Thus, there is a clinical demand for sensitive biomarker(s) to gauge the response to ICIs and beyond.

Talimogene laherparepvec (T-VEC) is a modified oncolytic herpes simplex virus expressing granulocyte-macrophage colony-stimulating factor promoting a local and systemic antitumor immune response.[3] As hypofractionated radiation (HRT) is more immunogenic than conventional radiation,[4] there is a strong rationale to combine T-VEC and HRT, especially in MCCs progressed on ICIs.

Cell-free circulating tumor DNA (ctDNA) has emerged as an important tool for monitoring molecular residual disease (MRD), owing to its minimal invasiveness and high concordance between genetic alternations detected in tumor and ctDNA.[5] Recent studies show that ctDNA is sensitive and effective in postoperative management, early detection of relapse, and prediction of treatment response in several human cancers.[6-9] In this pilot observation, we first demonstrate that a personalized and tumor-informed (bespoke) ctDNA assay is predictive of MCC treatment response. Moreover, combinatorial T-VEC and HRT is an effective alternative for an MCC patient progressed on pembrolizumab.

Abbreviations used:

CT:	computed tomography
CtDNA:	cell-free circulating tumor DNA
HRT:	hypofractionated radiation
ICIs:	immune checkpoint inhibitors
MCC:	Merkel cell carcinoma
MTM:	mean tumor molecules
T-VEC:	talimogene laherparepvec

CASE REPORT

A 70-year-old woman with MCC of the left wrist (Fig 1) and lymph node metastasis developed progressive disease and severe side effects on pembrolizumab. Despite additional subsequent surgery and radiation, she developed in-transit metastasis of the left arm (Table I). Treatment with HRT and T-VEC was initiated. A total of 8 T-VEC injections were administered to left forearm lesions in a standard schedule concurrently with HRT (a total of 30 Gy in 5 fractions). During the treatment course, she developed lesions on the left wrist (opposite side of the primary tumor) and left neck lymph node, which were both treated and resolved with HRT (Fig 2, *A* and *B*).

Response and disease burden was monitored by a periodic bespoke ctDNA assay (Signatera

From the Department of Radiation Oncology,[a] Department of Dermatology,[b] and Department of Surgery,[d] University of California, Irvine; Natera, Inc, Austin[c]; Veterans Affairs Long Beach Healthcare System[e]; and Southern California Institute for Research and Education, Long Beach.[f]

Funding sources: None.

IRB approval status: We have obtained informed consent to publish information and images from our patient.

Correspondence to: Ling Gao, MD, PhD, VA Long Beach Healthcare System, Department of Dermatology, University of California, 5901 E 7th St, Long Beach, CA 90822. E-mail: lingg3@uci.edu.

JAAD Case Reports 2021;18:94-8.
2352-5126

https://doi.org/10.1016/j.jdcr.2021.10.025

Table I. Summary of clinical events and treatment history

Date	Clinical event	Treatment
12/2018	Stage IIIb	WLE and SLNB (1/1)
4/2019	Relapse in left axillary lymph node	Pembrolizumab (Q3 weeks, 8 cycles)
7/2019	Generalized psoriasiform dermatitis and worsening rheumatoid arthritis	Topical steroids, NSAIDs
10/2019	Progression in left axillary lymph node	—
12/2019	—	Lymph node dissection
3/2020	—	Completion of radiation
5/6/2020	Patient noted new lesions on left arm	—
5/19/2020	In-transit metastasis	Biopsy performed
	Day 1	ctDNA #1 (0.25 MTM/mL)
6/9/2020	Day 22	T-VEC #1
6/29/2020	Day 42	ctDNA #2 (42.45 MTM/mL)
6/30/2020	Day 43	T-VEC #2
		HRT (left forearm)
7/14/2020	Day 57	ctDNA #3 (2.44 MTM/mL)
		T-VEC #3
7/28/2020	Day 71	T-VEC #4
8/11/2020	Day 85	T-VEC #5
8/18/2020	Day 92	HRT (left neck, left wrist)
8/25/2020	Day 99	T-VEC #6
9/8/2020	Day 113	T-VEC #7
9/15/2020	Day 120	ctDNA #4 (0 MTM/mL)
9/22/2020	Day 127	ctDNA #5 (0 MTM/mL)
		T-VEC #8
8/2/2021	Day 441	ctDNA #12 (0 MTM/mL)

ctDNA, Cell-free circulating tumor DNA; *HRT*, hypofractionated radiation; *MTM*, mean tumor molecules; *NSAIDs*, non-steroidal anti-inflammatory drugs; *SLNB*, sentinel lymph node biopsy; *T-VEC*, talimogene laherparepvec; *WLE*, wide local excision.

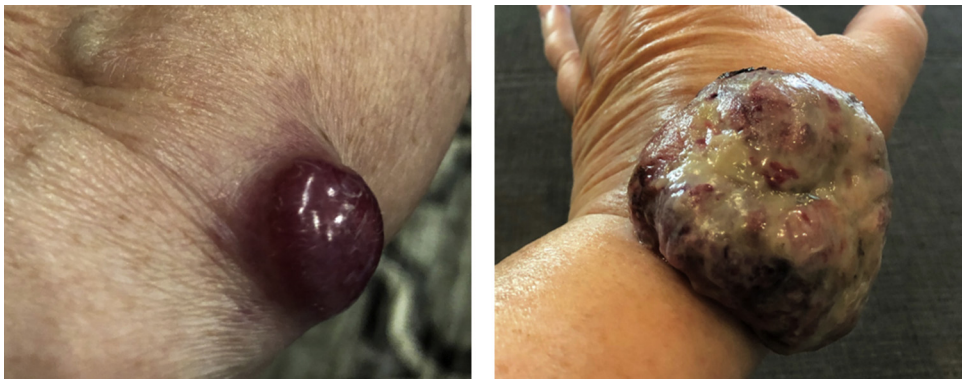

Fig 1. Aggressive primary Merkel cell carcinoma (MCC) on left wrist. Left panel: Photo taken prior to biopsy. Right panel: Photo taken 6 weeks after biopsy.

molecular residual disease test). Briefly, tumor-specific single nucleotide variants identified by whole exome sequencing (>20,000 genes) were tracked in plasma ctDNA by ultra-deep sequencing (median target coverage, ≥ ×105,000).[7] As demonstrated in Fig 3, in-transit metastasis was corroborated by initial positive ctDNA (0.25 mean tumor molecules [MTM]/mL), consistent with clinical observation; a 170-fold increase in ctDNA (42.45 MTM/mL) was detected on day 42, and ctDNA dropped to 2.44 MTM/mL after completion of HRT. Notably, 1 week after the seventh T-VEC injection, ctDNA had cleared (0 MTM/mL) and has remained undetectable since, the results have been supported by standard imaging studies. Based on serial negative ctDNA, the patient has been off any treatment with no evidence of recurrence through day 441 (Fig 3).

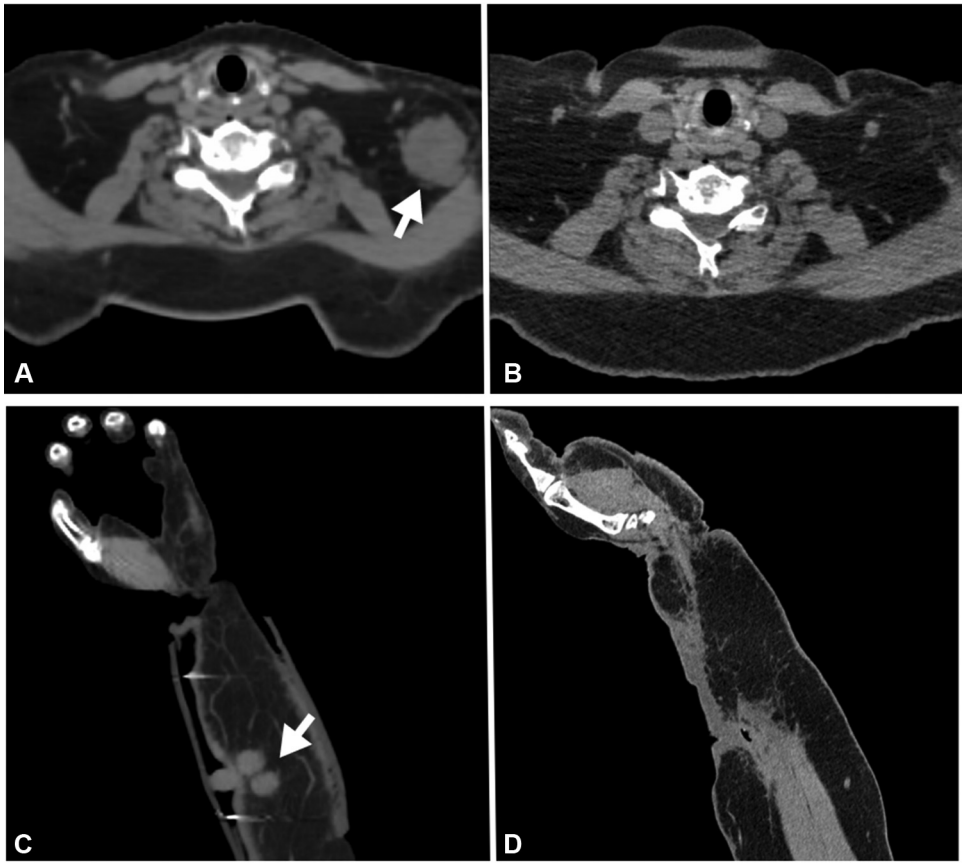

Fig 2. Pre- and post-treatment computed tomography (CT) imaging. **A**, Pre-treatment CT of the neck showing large left supraclavicular lymph node metastasis (*arrow*). **B**, Post-treatment CT of the neck. **C**, Pre-treatment CT of the left forearm showing cutaneous metastases on volar surface of forearm (*arrow*). **D**, Post-treatment CT of the left forearm.

DISCUSSION

Despite the approval of ICIs for metastatic MCC, primary and secondary resistance is common.[2] Proposed biomarkers of response such as Merkel cell polyomavirus, programmed death-ligand 1 expression, and mutation burden have not been productive, heralding the search for predictive biomarkers to gauge response, detect molecular residual disease, and early relapse.

Recently, detection of ctDNA via routine blood draws has gained widespread attention, owing to its obvious advantage as a minimally invasive procedure and the ease of longitudinal blood collections. In contrast to serum surrogate markers and circulating tumor cell counts,[10] bespoke ctDNA directly measures tumor-informed somatic variants. Compared with assays tracking only major genetic mutations, bespoke ctDNA leverages whole exome sequencing, greatly increasing detection sensitivity with broader application for all MCCs.[8] Moreover, bespoke ctDNA has been proven as an effective predictive biomarker in solid tumors treated with pembrolizumab.[6] Gold-standard imaging studies have feasibility challenges, including insurance coverage and test sensitivity. In patients with urothelial bladder carcinoma, rising ctDNA levels preceded radiographic changes, suggesting that ctDNA can be used as a sensitive and cost-effective early detection method of relapse and disease surveillance complementary to radiographic studies.[7] ctDNA levels detected in our patient consistently showed high concordance with clinical observations and findings by imaging studies.

The standard of care for stage I-III MCC is surgery in conjunction with radiation. Currently, it is impossible to determine which patients harbor residual disease after treatment with curative intent. As immunotherapy in the adjuvant setting is currently under clinical investigation (NCT03271372, NCT03712605), ctDNA could stratify patients who are likely to benefit, while sparing others from unnecessary costs and potential side effects.

Recently, T-VEC has been used alone or with ICIs in locally advanced MCC patients.[9] T-VEC has a dual mechanism of action, an oncolytic effect, whereby it

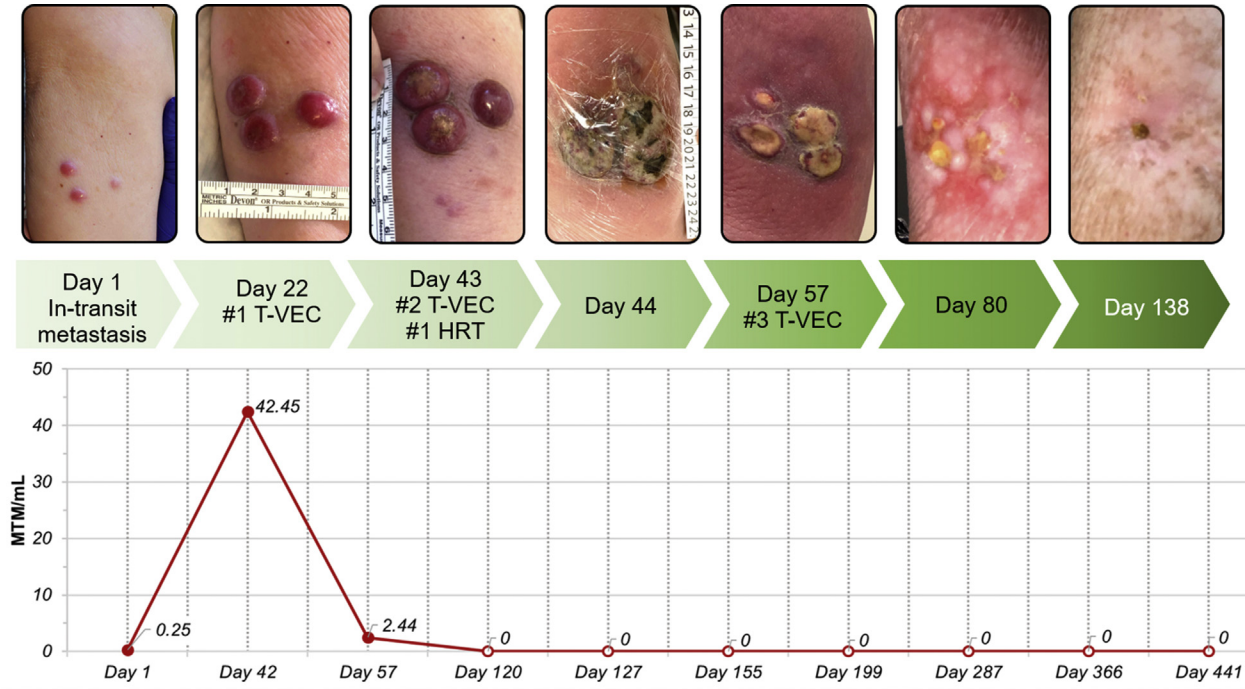

Fig 3. Timeline of treatment course and cell-free circulating tumor DNA (ctDNA) analysis. Photos (top) demonstrate lesion appearance during treatment events (middle), correlated to line graph (bottom) of bespoke ctDNA (Signatera molecular residual disease test) results over the course. Event dates are presented as days since biopsy of in-transit metastases (defined as day 1). Talimogene laherparepvec (T-VEC) injection was initiated on day 22 and last dose was administered on day 127. ctDNA levels are measured in mean tumor molecules (MTM) per milliliter (mL). In-transit metastasis was corroborated by initial positive ctDNA (0.25 MTM/mL) and increasing tumor burden correlated with a 170-fold increase in ctDNA (42.45 MTM/mL) on day 42 prior to the second T-VEC injection and day 1 hypofractionated radiation. On day 57, ctDNA decreased to 2.44 MTM/mL at the time of the third T-VEC injection and after completion of the first hypofractionated radiation. ctDNA levels cleared (0 MTM/mL) 1 week after the seventh T-VEC injection (day 120) and has remained undetectable through day 441.

directly infects and kills local tumor cells at the injection site, as well as an immunotherapy effect through induction of local and systemic immune responses. However, to date there is no efficacy report of the combined use of T-VEC and HRT for MCC progressed on ICIs. In our patient, we have first demonstrated that this regimen is effective and could be an alternative for patients who develop severe side effects and who are resistant to ICIs. Given the aggressive nature of our patient's MCC, which recurred shortly after every prior treatment modality, this is a promising outcome.

In this pilot observation, we have illustrated that bespoke ctDNA analysis is predictive for MCC treatment response and surveillance. Moreover, the cost efficacy renders an attractive opportunity for close monitoring. Considering the challenges posed by COVID-19, routine blood draws can even be achieved in patients' homes, thus minimizing potential exposure. Further studies are warranted to define the clinical implications of ctDNA in postoperative risk stratification, early detection of relapse, and treatment response monitoring. Our study highlights the potentially transformative role of ctDNA in clinical decision making, and we argue that such objectives are achievable in MCC, as current technologies and real-world evidence continue to mount.

We thank Dr John Fruehauf for his excellent care of the patient, and Dr Fariba Jafari, Authors Elizabeth Sanzaro and Bovey Zhou at Natera for the support.

Conflicts of interest

LG was a consultant for EMD Serono (2015) and received research support from Gilead (2015-2017). NHR and AA are employees of Natera, Inc; AA has equity ownership in Mission Bio and Notable Labs. MY received research support from Castle Biosciences. JY, AK, JPH have no disclosures.

REFERENCES

1. Paulson KG, Park SY, Vandeven NA, et al. Merkel cell carcinoma: Current US incidence and projected increases based on changing demographics. *J Am Acad Dermatol*. 2018;78:457-463.e2.
2. Gallo M, Guarnotta V, De Cicco F, et al. Immune checkpoint blockade for Merkel cell carcinoma: Actual findings and unanswered questions. *J Cancer Res Clin Oncol*. 2019;145(2): 429-443.
3. Jhawar SR, Goyal S, Thandoni A, et al. Radiation therapy and talimogene laherparepvec (TVEC) synergize in the treatment of melanoma. *J Immunol*. 2017;198(1): 79.32.
4. Frey B, Rückert M, Weber J, et al. Hypofractionated irradiation has immune stimulatory potential and induces a timely restricted infiltration of immune cells in colon cancer tumors. *Front Immunol*. 2017;8:231.
5. Kilgour E, Rothwell DG, Brady G, Dive C. Liquid biopsy-based biomarkers of treatment response and resistance. *Cancer Cell*. 2020;37:485-495.
6. Bratman SV, Yang SYC, Iafolla MAJ, et al. Personalized circulating tumor DNA analysis as a predictive biomarker in solid tumor patients treated with pembrolizumab. *Nat Cancer*. 2020;1:873-881.
7. Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free DNA in patients with urothelial bladder carcinoma. *J Clin Oncol*. 2019; 37(18):1547-1557.
8. Reinert T, Henriksen TV, Christensen E, et al. Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. *JAMA Oncol*. 2019;5(8):1124-1131.
9. Westbrook BC, Norwood TG, Terry NLJ, McKee SB, Conry RM. Talimogene laherparepvec induces durable response of regionally advanced Merkel cell carcinoma in 4 consecutive patients. *JAAD Case Rep*. 2019;5(9):782-786.
10. Gaiser MR, Daily K, Hoffmann J, Brune M, Enk A, Brownell I. Evaluating blood levels of neuron specific enolase, chromogranin A, and circulating tumor cells as Merkel cell carcinoma biomarkers. *Oncotarget*. 2015;6(28):26472-26482.