



Promise of hypoxia-targeted tracers in metastatic lymph node imaging

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Accurate identification and characterization of lymph node metastases (LNM) has important therapeutic and prognostic implications for most malignancies, with the site and number of involved LNs having a directive value on patient staging, treatment selection and planning, and eventual treatment outcomes [1, 2]. Clinically, detection of LNMs relies on invasive biopsy procedures. A combination of ^{99m}Tc colloids and optical dye is typically used to guide SLN biopsy, followed by pathological assessment of the excised LNs [3]. The success of this method relies on quantity and quality of biopsied LN samples and comprehensive microscopic inspections of entire LN volumes which is unrealistic and prone to inter-operator variability. Noninvasive imaging methods, primarily, computed tomography (CT), magnetic resonance imaging (MRI), or positron emission tomography (PET) are also used routinely in the clinic and are complementary to biopsy procedures [4]. However, each method is fraught with its own set of limitations—structural imaging modalities such as MRI and CT rely on nodal size and morphology with limited sensitivity and specificity. The presence of necrosis on MRI is another commonly used indicator of LNMs. However, it suffers from inter-observer variability

and demonstrates poor negative predictive value, particularly for non-enlarged and smaller nodes [4]. Functional MRI methods that evaluate diffusion and perfusion suffer from drawbacks such as suboptimal signal-to-noise ratio and poor spatial resolution, which impacts both specificity and sensitivity of diagnoses. Dynamic contrast-enhanced (DCE) MRI that assesses vascularity has also demonstrated moderate success (72% sensitivity and 87% specificity) in LNM identification [2]. Molecular imaging methods such as PET may overcome some of these challenges and provide substantially improved sensitivity and specificity over structural imaging modalities. [18F]FDG-PET is a widely used tool in PET imaging of sentinel LNs, demonstrating high sensitivity (~92–100%) and mixed specificity (77–93%), although recent reports have demonstrated limited specificity in differentiating LNMs from inflamed LNs [1, 5]. However, the biggest challenge with PET imaging of LNMs lies in the limited spatial resolution: ~8–10 mm in conventional whole-body scanners and 4–5 mm in specially designed organ-specific scanners. Thus, it is somewhat unrealistic to rely on PET for detection of early LNMs, occult metastases, and extracapsular and extranodal extensions (ENE) within the LNs. Other targeted radiotracers such as [68 Ga]PSMA have also been used in LNM staging in prostate cancer, but non-specific uptake in adjacent ganglia was demonstrated to be a major pitfall [1].

Hypoxia is an attractive target for LNM imaging and staging owing to its well-established role as a negative prognostic factor in malignancies such as head and neck, breast, pancreatic, and prostate cancers. Hypoxic micro-environments result from aberrant tumor metabolism and dysregulated vascular supply; are associated with tumor progression, invasion, and metastasis; and lead to treatment failure in chemo-, radiation, and immunotherapies [6, 7]. Locally advanced and recurrent cancers show enhanced hypoxia at the primary site as well as in the draining lymphatic nodes [6, 8]. Traditional methods of hypoxia measurement rely on invasive polarographic methods in

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accessible primary tumors or LNs. These methods lack spatial information and repeatability, limiting their clinical utility. In contrast, noninvasive LNM imaging with hypoxia-targeting agents such as [18F]fluoromisonidazole (FMISO) and [18F]fluoroazomycinaraboside (FAZA) PET provides quantitative, spatial, and longitudinal imaging capabilities and is being evaluated in various clinical trials, across a broad spectrum of cancers [9–11]. Similarly, bivalent [$^{60/61/62/64}\text{Cu}$]Cu-labeled diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) has emerged as a promising hypoxia tracer. However, only limited studies have evaluated the potential of Cu-ATSM in LNM staging [9, 12]. The use of [18F]FDG as a surrogate biomarker of hypoxia has been proposed owing to HIF-driven upregulation of glucose transporters (GLUTs) and glycolytic enzymes. However, recent studies have found that tumor cells shift their metabolism towards fatty acid oxidation to survive in LN microenvironments [13]. Thus, the utility of [18F]FDG in hypoxia-targeted LNM identification and staging may be limited, highlighting the need for more specific tracers.

Other hypoxia-specific molecular tracers targeting aberrant tumor metabolism have primarily focused on upregulated transcription factors of hypoxia-inducible factors 1 and 2 (HIF 1 and 2). Carbonic anhydrase IX (CAIX), a transmembrane enzyme involved in the cellular regulation of pH homeostasis and a downstream target of HIF-1 α , has emerged as a promising target for hypoxia imaging [14]. With the exception of renal cell carcinoma, CAIX expression is constitutively upregulated in and strongly correlated with reduced oxygenation status in primary and metastatic sites in several tumor types [15]. CAIX gene expression is associated with poor prognosis and is upregulated in LNMs of patients with breast and cervical cancer, oral cavity squamous cell carcinoma, melanoma, and as demonstrated by Tian et al. in this issue of the *European Journal of Nuclear Medicine and Molecular Imaging*, in occult LNMs and ENEs of primary and recurrent nasopharyngeal carcinomas [15–21]. Thus, development of CAIX-specific diagnostic and therapeutic agents is emerging as a promising strategy to target hypoxic and metastatic lymph nodes.

The Grawitz250 (G250) monoclonal antibody (mAb) developed in the 1980s by Oosterwijk et al. was the first tracer targeting CAIX in clear cell renal cell carcinoma [22]. Since then, a number of studies have reported the application of chimeric version of G250 (cG250), other mAbs, and antibody fragments towards CAIX-targeted imaging [15, 23]. PET and optical imaging probes based on these antibody and antibody fragments have shown excellent selectivity for LNMs in preclinical investigations [16, 24–27]. A potential concern with mAb and mAb-based tracers stems from the relatively stable expression (half-life ~ 40 h) of CAIX, with the enzyme remaining on the cell surface even after

reoxygenation, potentially increasing the percentage of false-positive findings [15, 28].

Among the small molecule tracers for CAIX targeting, acetazolamide derivatives are the best investigated class of inhibitors demonstrating high affinity, availability, and ease of chemical modification [23, 29]. Agents such as $^{99\text{m}}\text{Tc}$ -PHC-102 have shown promise in the clinic [30, 31]. Interestingly, in vitro studies have shown that these inhibitors only bind the active CAIX enzyme under hypoxic conditions [28]. The ability to distinguish cells that are currently hypoxic from those that are not, brings an important advantage to the sulfonamide inhibitor-based imaging agents when compared to mAbs. To further improve CAIX binding, dual motif ligands based on acetazolamide have been developed (first reported by Scheuermann and coworkers), demonstrating subnanomolar binding affinity and dramatically enhanced tumor targeting in vivo [32]. The superior CAIX targeting capacity afforded by the dual binding motif can be especially valuable in capturing small and clinically undetectable LNMs which is critical for nodal staging and prognostication. The ligands have been broadly employed as templates for PET, optical, and photoacoustic imaging tracers in various primary tumor xenograft models; however, their application in LNM staging has only recently been explored [33–35].

In the present study, Tian and coworkers report a fluorescence molecular tomography (FMT) and ultrasound-guided photoacoustic imaging (US-PA) strategy for detection of occult LNMs and ENEs in mouse models of nasopharyngeal carcinomas (NPC) using the dual motif CAIX ligand conjugated to IRDye800 (CAIX-800) [21]. Fluorescence imaging with CAIX-800 could detect small nodal metastases with diameter less than 5 mm and volume range from 3 to 15 mm³ with sensitivity of 81.3% and specificity of 93.8%. By comparison, [18F]FDG demonstrates poor sensitivity in detecting nodal metastases with volumes less than 80 mm³ (less than 23% for LNMs less than 5 mm) [1, 36]. Impressively, CAIX-800 could differentiate inflammatory from metastatic LNMs, as a further advantage over [18F]FDG PET. US-PA imaging using the same construct enabled sensitive detection of pathological ENEs and micrometastatic LNMs. On comparison with conventional T2-weighted MRI and ultrasound, US-PA with CAIX-800 demonstrated superior sensitivity in early detection of occult LNMs. US-PA could also noninvasively visualize and track pathological progression of ENEs through CAIX-800 signal in the nodal periphery, which was microscopically validated both by H&E and CAIX staining [21].

Although it remains to be tested in the clinical settings and several questions remain, these findings open a promising avenue for hypoxia-based imaging tracers in LNM detection and staging. To adequately assess the sensitivity for detection of early LNM and ENEs, it would be crucial to test CAIX-800 in more representative models of spontaneous

LNM arising from orthotopic tumors. Models arising from footpad injections may not truly represent the sparse number of cancer cells in LNM which may significantly impact the overall sensitivity and specificity of detection. Evaluating the specificity of the agent for CAIX compared to other isoforms of CA family of receptors, particularly CAXII and its impact on LNM staging, is another critical issue that needs attention before clinical translation can be envisaged. Cross-reactivity with CAXII expression could be a confounding factor, although this stringent measure of selectivity may not be as important in detection of LNMs and disseminated disease [15]. It would be interesting to assess the utility of CAIX-800 as a companion diagnostic in treatment guidance and monitoring response to chemo-, radiation, and immune therapies, where hypoxia is a well-established defining factor of treatment outcomes. Although these studies were performed in NPC mouse models, CAIX-800 may be broadly applicable to LNMs of other malignancies as well, given the overexpression of CAIX in several aggressive and recurrent cancers. This significantly multiplies the translational and clinical impact of CAIX-800.

US-PA and FMT imaging technologies are slowly being adopted in the clinic as viable approaches for LNM staging and resection [37–40]. The limited depth of penetration and relatively poor quantification afforded by these optical technologies severely hampers their widespread use. In particular, assessment of trunk nodes, which are the most frequent site of LNMs for many cancers, may be difficult with optical technologies. Thus, concerted efforts towards optical imaging technology improvement are needed. However, the assessment of occult LNMs for relatively superficially situated tumors such as NPC, head and neck tumors, breast cancers, and melanomas is readily achievable [39, 40]. Radiation-free and user-friendly workflows with optical imaging technologies may allow for more frequent LNM staging, which is currently not possible by conventional imaging modalities, leading to improved prognostication and treatment planning in the clinic, at least for a subset of cancer types. Further, multimodal imaging workflows combining complementary imaging modalities, coupled with multimodal molecularly targeted tracers, may serve to significantly enhance the diagnostic accuracy and sensitivity in occult LNM detection [3, 41].

Overall, imaging of occult and early stage LNMs is critical and challenging and, therefore, an area of intense research interest both in the preclinical and clinical domains. Metastases are a major cause of cancer-related deaths and the establishment of LNM plays a key role in initiating the cascade [42]. Development of sensitive tracers that can detect early LNMs and noninvasively map their longitudinal progression is crucial not only for improving the clinical management of the disease but also in advancing our understanding of the biology and sequelae of the metastatic

process. Since hypoxia is a ubiquitous feature of most cancers and plays an important role in the initiation and progress of the metastatic disease in locally advanced and recurrent cancers, the combination of enhanced noninvasive imaging methods, specific and sensitive molecular tracers, and robust animal models can potentially address this challenging issue.

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Declarations

Studies with human participants or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

References

1. Alavi A, Carlin SD, Werner TJ, Al-Zaghal A. Suboptimal sensitivity and specificity of PET and other gross imaging techniques in assessing lymph node metastasis. *Mol Imaging Biol*. 2019;21:808–11. <https://doi.org/10.1007/s11307-018-01311-4>.
2. Chen J, Hagiwara M, Givi B, Schmidt B, Liu C, Chen Q, et al. Assessment of metastatic lymph nodes in head and neck squamous cell carcinomas using simultaneous 18F-FDG-PET and MRI. *Sci Rep*. 2020;10:20764. <https://doi.org/10.1038/s41598-020-77740-5>.
3. KleinJan GH, van Werkhoven E, van den Berg NS, Karakullukcu MB, Zijlman HJMAA, van der Hage JA, et al. The best of both worlds: a hybrid approach for optimal pre- and intraoperative identification of sentinel lymph nodes. *Eur J Nucl Med Mol Imaging*. 2018;45:1915–25. <https://doi.org/10.1007/s00259-018-4028-x>.
4. Kim JH, Choi KY, Lee S-H, Lee DJ, Park BJ, Yoon DY, et al. The value of CT, MRI, and PET-CT in detecting retropharyngeal lymph node metastasis of head and neck squamous cell carcinoma. *BMC Med Imaging*. 2020;20:88. <https://doi.org/10.1186/s12880-020-00487-y>.
5. Houshmand S, Salavati A, Segtnan EA, Grupe P, Høiland-Carlson PF, Alavi A. Dual-time-point imaging and delayed-time-point fluorodeoxyglucose-PET/computed tomography imaging in various clinical settings. *PET Clin*. 2016;11:65–84. <https://doi.org/10.1016/j.cpet.2015.07.003>.
6. Rankin EB, Giaccia AJ. Hypoxic control of metastasis. *Science*. 2016;352:175–80. <https://doi.org/10.1126/science.aaf4405>.
7. Van den Eynden GG, Van der Auwera I, Van Laere SJ, Colpaert CG, Turley H, Harris AL, et al. Angiogenesis and hypoxia in lymph node metastases is predicted by the angiogenesis and hypoxia in the primary tumour in patients with breast cancer. *Br J Cancer*. 2005;93:1128–36. <https://doi.org/10.1038/sj.bjc.6602828>.

8. Firmino NS, Cederberg RA, Lee CM, Shi R, Wadsworth BJ, Franks SE, et al. Germinal center hypoxia in tumor-draining lymph nodes negatively regulates tumor-induced humoral immune responses in mouse models of breast cancer. *Oncoimmunology*. 2021;10:1959978. <https://doi.org/10.1080/2162402x.2021.1959978>.
9. Lopci E, Grassi I, Chiti A, Nanni C, Cicoria G, Toschi L, et al. PET radiopharmaceuticals for imaging of tumor hypoxia: a review of the evidence. *Am J Nucl Med Mol Imaging*. 2014;4:365–84.
10. Capitanio U, Pepe G, Incerti E, Larcher A, Trevisani F, Lucianò R, et al. The role of 18F-FAZA PET/CT in detecting lymph node metastases in renal cell carcinoma patients: a prospective pilot trial. *Eur J Nucl Med Mol Imaging*. 2021;48:554–60. <https://doi.org/10.1007/s00259-020-04936-2>.
11. Bandurska-Luque A, Löck S, Haase R, Richter C, Zöphel K, Perin R, et al. Correlation between FMISO-PET based hypoxia in the primary tumour and in lymph node metastases in locally advanced HNSCC patients. *Clin Transl Radiat Oncol*. 2019;15:108–12. <https://doi.org/10.1016/j.ctro.2019.02.002>.
12. Lohith TG, Kudo T, Demura Y, Umeda Y, Kiyono Y, Fujibayashi Y, et al. Pathophysiologic correlation between 62Cu-ATSM and 18F-FDG in lung cancer. *J Nucl Med*. 2009;50:1948–53. <https://doi.org/10.2967/jnumed.109.069021>.
13. Lee CK, Jeong SH, Jang C, Bae H, Kim YH, Park I, et al. Tumor metastasis to lymph nodes requires YAP-dependent metabolic adaptation. *Science*. 2019;363:644–9. <https://doi.org/10.1126/science.aav0173>.
14. Lau J, Lin KS, Bénard F. Past, present, and future: development of theranostic agents targeting carbonic anhydrase IX. *Theranostics*. 2017;7:4322–39. <https://doi.org/10.7150/thno.21848>.
15. Tafreshi NK, Lloyd MC, Bui MM, Gillies RJ, Morse DL. Carbonic anhydrase IX as an imaging and therapeutic target for tumors and metastases. *Subcell Biochem*. 2014;75:221–54. https://doi.org/10.1007/978-94-007-7359-2_12.
16. Huizing FJ, Garousi J, Lok J, Franssen G, Hoebe BAW, Frejd FY, et al. CAIX-targeting radiotracers for hypoxia imaging in head and neck cancer models. *Sci Rep*. 2019;9:18898. <https://doi.org/10.1038/s41598-019-54824-5>.
17. Brockton NT, Klimowicz AC, Bose P, Petrillo SK, Konno M, Rudmik L, et al. High stromal carbonic anhydrase IX expression is associated with nodal metastasis and decreased survival in patients with surgically-treated oral cavity squamous cell carcinoma. *Oral Oncol*. 2012;48:615–22. <https://doi.org/10.1016/j.oraloncology.2012.01.018>.
18. Ong CHC, Lee DY, Lee B, Li H, Lim JCT, Lim JX, et al. Hypoxia-regulated carbonic anhydrase IX (CAIX) protein is an independent prognostic indicator in triple negative breast cancer. *Breast Cancer Res*. 2022;24:38. <https://doi.org/10.1186/s13058-022-01532-0>.
19. Choschzick M, Oosterwijk E, Müller V, Woelber L, Simon R, Moch H, et al. Overexpression of carbonic anhydrase IX (CAIX) is an independent unfavorable prognostic marker in endometrioid ovarian cancer. *Virchows Arch*. 2011;459:193–200. <https://doi.org/10.1007/s00428-011-1105-y>.
20. Lee S, Shin HJ, Han IO, Hong EK, Park SY, Roh JW, et al. Tumor carbonic anhydrase 9 expression is associated with the presence of lymph node metastases in uterine cervical cancer. *Cancer Sci*. 2007;98:329–33. <https://doi.org/10.1111/j.1349-7006.2007.00396.x>.
21. Huang W, Wang K, Huang W, et al. Carbonic anhydrase IX stratifies patient prognosis and identifies nodal status in animal models of nasopharyngeal carcinoma using a targeted imaging strategy. *Eur J Nucl Med Mol Imaging*. 2022. <https://doi.org/10.1007/s00259-022-05922-6>.
22. Oosterwijk E, Ruiter DJ, Hoedemaeker PJ, Pauwels EK, Jonas U, Zwartendijk J, et al. Monoclonal antibody G 250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. *Int J Cancer*. 1986;38:489–94. <https://doi.org/10.1002/ijc.2910380406>.
23. Kciuk M, Gielecińska A, Mujwar S, Mojzych M, Marciniak B, Drozd R, et al. Targeting carbonic anhydrase IX and XII isoforms with small molecule inhibitors and monoclonal antibodies. *J Enzyme Inhib Med Chem*. 2022;37:1278–98. <https://doi.org/10.1080/14756366.2022.2052868>.
24. Verhoeff SR, van Es SC, Boon E, van Helden E, Angus L, Elias SG, et al. Lesion detection by [(89)Zr]Zr-DFO-girentuximab and [(18)F]FDG-PET/CT in patients with newly diagnosed metastatic renal cell carcinoma. *Eur J Nucl Med Mol Imaging*. 2019;46:1931–9. <https://doi.org/10.1007/s00259-019-04358-9>.
25. van Lith SAM, Huizing FJ, Franssen GM, Hoebe BAW, Lok J, Doukeridou S, et al. Novel VHH-based tracers with variable plasma half-lives for imaging of CAIX-expressing hypoxic tumor cells. *Mol Pharm*. 2022. <https://doi.org/10.1021/acs.molpharmaceut.1c00841>.
26. Huizing FJ, Hoebe BAW, Franssen GM, Boerman OC, Heskamp S, Bussink J. Quantitative imaging of the hypoxia-related marker CAIX in head and neck squamous cell carcinoma xenograft models. *Mol Pharm*. 2019;16:701–8. <https://doi.org/10.1021/acs.molpharmaceut.8b00950>.
27. Tafreshi NK, Bui MM, Bishop K, Lloyd MC, Enkemann SA, Lopez AS, et al. Noninvasive detection of breast cancer lymph node metastasis using carbonic anhydrases IX and XII targeted imaging probes. *Clin Cancer Res*. 2012;18:207–19. <https://doi.org/10.1158/1078-0432.Ccr-11-0238>.
28. Švastová E, Hulíková A, Rafajová M, Zat'ovičová M, Gibadulinová A, Casini A, et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. *FEBS Lett*. 2004;577:439–45. <https://doi.org/10.1016/j.febslet.2004.10.043>.
29. Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. *Nat Rev Drug Discov*. 2008;7:168–81. <https://doi.org/10.1038/nrd2467>.
30. Krall N, Pretto F, Mattarella M, Müller C, Neri D. A 99mTc-labeled ligand of carbonic anhydrase IX selectively targets renal cell carcinoma in vivo. *J Nucl Med*. 2016;57:943–9. <https://doi.org/10.2967/jnumed.115.170514>.
31. Kulterer OC, Pfaff S, Wadsak W, Garstka N, Remzi M, Vraká C, et al. A microdosing study with (99m)Tc-PHC-102 for the SPECT/CT imaging of primary and metastatic lesions in renal cell carcinoma patients. *J Nucl Med*. 2021;62:360–5. <https://doi.org/10.2967/jnumed.120.245530>.
32. Wichert M, Krall N, Decurtins W, Franzini RM, Pretto F, Schneider P, et al. Dual-display of small molecules enables the discovery of ligand pairs and facilitates affinity maturation. *Nat Chem*. 2015;7:241–9. <https://doi.org/10.1038/nchem.2158>.
33. Huang W, Wang K, An Y, Meng H, Gao Y, Xiong Z, et al. In vivo three-dimensional evaluation of tumour hypoxia in nasopharyngeal carcinomas using FMT-CT and MSOT. *Eur J Nucl Med Mol Imaging*. 2020;47:1027–38. <https://doi.org/10.1007/s00259-019-04526-x>.
34. Minn I, Koo SM, Lee HS, Brummet M, Rowe SP, Gorin MA, et al. [64Cu]XYIMSR-06: a dual-motif CAIX ligand for PET imaging of clear cell renal cell carcinoma. *Oncotarget*. 2016;7:56471–9. <https://doi.org/10.18632/oncotarget.10602>.
35. Yang X, Minn I, Rowe SP, Banerjee SR, Gorin MA, Brummet M, et al. Imaging of carbonic anhydrase IX with an 111In-labeled dual-motif inhibitor. *Oncotarget*. 2015;6:33733–42. <https://doi.org/10.18632/oncotarget.5254>.
36. Kyzas PA, Evangelou E, Denaxa-Kyza D, Ioannidis JP. 18F-fluorodeoxyglucose positron emission tomography to evaluate cervical node metastases in patients with head and neck squamous cell carcinoma: a meta-analysis. *J Natl Cancer Inst*. 2008;100:712–20. <https://doi.org/10.1093/jnci/djn125>.

37. Zeng H-C, Hu J-L, Bai J-W, Zhang G-J. Detection of sentinel lymph nodes with near-infrared imaging in malignancies. *Mol Imaging Biol.* 2019;21:219–27. <https://doi.org/10.1007/s11307-018-1237-4>.
38. Nishio N, van den Berg NS, van Keulen S, Martin BA, Fakurnejad S, Teraphongphom N, et al. Optical molecular imaging can differentiate metastatic from benign lymph nodes in head and neck cancer. *Nat Commun.* 2019;10:5044. <https://doi.org/10.1038/s41467-019-13076-7>.
39. Ahuja AT, Ying M, Ho SY, Antonio G, Lee YP, King AD, et al. Ultrasound of malignant cervical lymph nodes. *Cancer Imaging.* 2008;8:48–56. <https://doi.org/10.1102/1470-7330.2008.0006>.
40. Stoffels I, Jansen P, Petri M, Goerdt L, Brinker TJ, Griewank KG, et al. Assessment of nonradioactive multispectral optoacoustic tomographic imaging with conventional lymphoscintigraphic imaging for sentinel lymph node biopsy in melanoma. *JAMA Netw Open.* 2019;2:e199020. <https://doi.org/10.1001/jamanetworkopen.2019.9020>.
41. Akers WJ, Edwards WB, Kim C, Xu B, Erpelding TN, Wang LV, et al. Multimodal sentinel lymph node mapping with single-photon emission computed tomography (SPECT)/computed tomography (CT) and photoacoustic tomography. *Transl Res.* 2012;159:175–81. <https://doi.org/10.1016/j.trsl.2011.09.006>.
42. Reticker-Flynn NE, Zhang W, Belk JA, Basto PA, Escalante NK, Pilarowski GOW, et al. Lymph node colonization induces tumor-immune tolerance to promote distant metastasis. *Cell.* 2022;185:1924–42.e23. <https://doi.org/10.1016/j.cell.2022.04.019>.

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