**Figure 3.**

Validation of the I/P ratio as a predictor of survival after DC vaccination. A, relative tumor size, size of peritumoral area, and CD3⁺ count of all patients in the validation cohort. Overall survival is shown above each tumor in months. Each large bullet point represents 1,000 T cells within the tumor (white) or in the peritumoral area (black). B, peritumoral (margin) and intratumoral (tumor) CD3⁺ T-cell densities (CD3⁺ T cells/mm²) shown separately for short and long survivors. C–E, correlation between I/P ratio and overall survival are shown on a log–log scale for all patients (C), patients with substage up to M1b (D), and patients with substage M1c (E). F, survival of patients with low (<1, $n = 33$) and high (>1, $n = 6$) I/P ratios ($P = 0.0011$, log-rank test). G, AUC analysis for discriminating short survivors (bottom 50% survival times) and long survivors (top 50%) using the I/P ratio (AUC of 0.88). H, comparison with two other relevant prognostic markers. A combined logistic regression model incorporating the ratio and LDH achieves an AUC of 0.95 (M substage alone: 0.68; LDH alone: 0.72), which is no longer improved by also taking the M substage into account.

Table 2. Multivariate analysis

	Log OR (95% CI)	P
Intercept	0.82 (0.05-1.6)	0.044
I/P ratio (log ₁₀)	0.4 (0.19-0.61)	0.001
M stage at inclusion	0 (-0.18-0.19)	0.97
LDH	-0.37 (-0.64 to -0.1)	0.013
Gender	0 (-0.3-0.31)	0.98
Months to M stage	0 (0-0.01)	0.54
Breslow thickness	0 (-0.06-0.06)	0.96
Age at inclusion	0 (-0.01-0.01)	0.83

NOTE: Logistic regression model showing that the difference in ratio in the validation cohort is not explained by including M substage, LDH, and other possible confounders as covariates.

compared with LDH (AUC = 0.72) and M substage (AUC = 0.68). Combining the I/P ratio with LDH in a logistic regression model, we even obtained an AUC value of 0.95. In a larger multivariate analysis (Table 2), the I/P ratio was the strongest predictor ($P = 0.001$; log OR, 0.4; 95% CI, 0.19–0.61) for survival in the validation cohort. LDH level remained an independent, but weaker predictor ($P = 0.013$; log OR, -0.37; 95% CI, -0.64 to -0.1), whereas M substage at start of DC-based immunotherapy, gender, Breslow thickness, age, and time to M stage did not show significant prognostic value. These multivariate analyses provide compelling evidence that strong T-cell infiltration in the primary melanoma is an independent predictor of survival in metastatic melanoma patients receiving DC-based immunotherapy, despite the fact that several years typically pass between resection of the primary tumor and the onset of treatment.

T-cell infiltration is a potential predictive biomarker for DC vaccination

The I/P ratio in primary tumors would be tremendously useful as a predictive biomarker for (adjuvant) treatment selection due to its availability at an early stage of disease. Establishing a biomarker requires analysis of data from a randomized controlled trial, which is not available yet for DC vaccinations worldwide; so far, most DC studies are small stage I/II studies varying from 10 to 40 patients. Therefore, as an approximation, we compared our DC-vaccinated cohorts to a cohort of patients receiving standard treatment (chemotherapy) in the same time-frame, matched on known prognostic factors to minimize confounding. While this approach cannot provide definitive proof of biomarker status, it did allow us to probe our hypothesis that patients require strong intratumoral T-cell infiltration to benefit from immunotherapy.

We analyzed 19 primary melanomas of patients that had received chemotherapy (Table 1; Fig. 4A). Survival was evaluated with Kaplan–Meier estimates comparing patients with low (<1 , $n = 6$) and high (>1 , $n = 13$) I/P ratios (Fig. 4B). Although the survival curves suggest that strong T-cell infiltration may correspond to longer survival in chemotherapy-treated patients, the difference is not significant ($P = 0.089$). There was also no appreciable direct correlation between survival and I/P ratio ($r = 0.12$, $P = 0.62$; Fig. 4C), thus, the I/P ratio is not a strong predictor of survival in chemotherapy-treated patients. This is in line with our hypothesis that the effect of T-cell infiltration in the primary tumor needs to be augmented by immunotherapy to improve survival. To further support this hypothesis, we matched each chemotherapy patient to two DC vaccination patients based on LDH level and M substage (Fig. 4D). DC-vaccinated patients with low I/P ratios showed no significant difference in survival

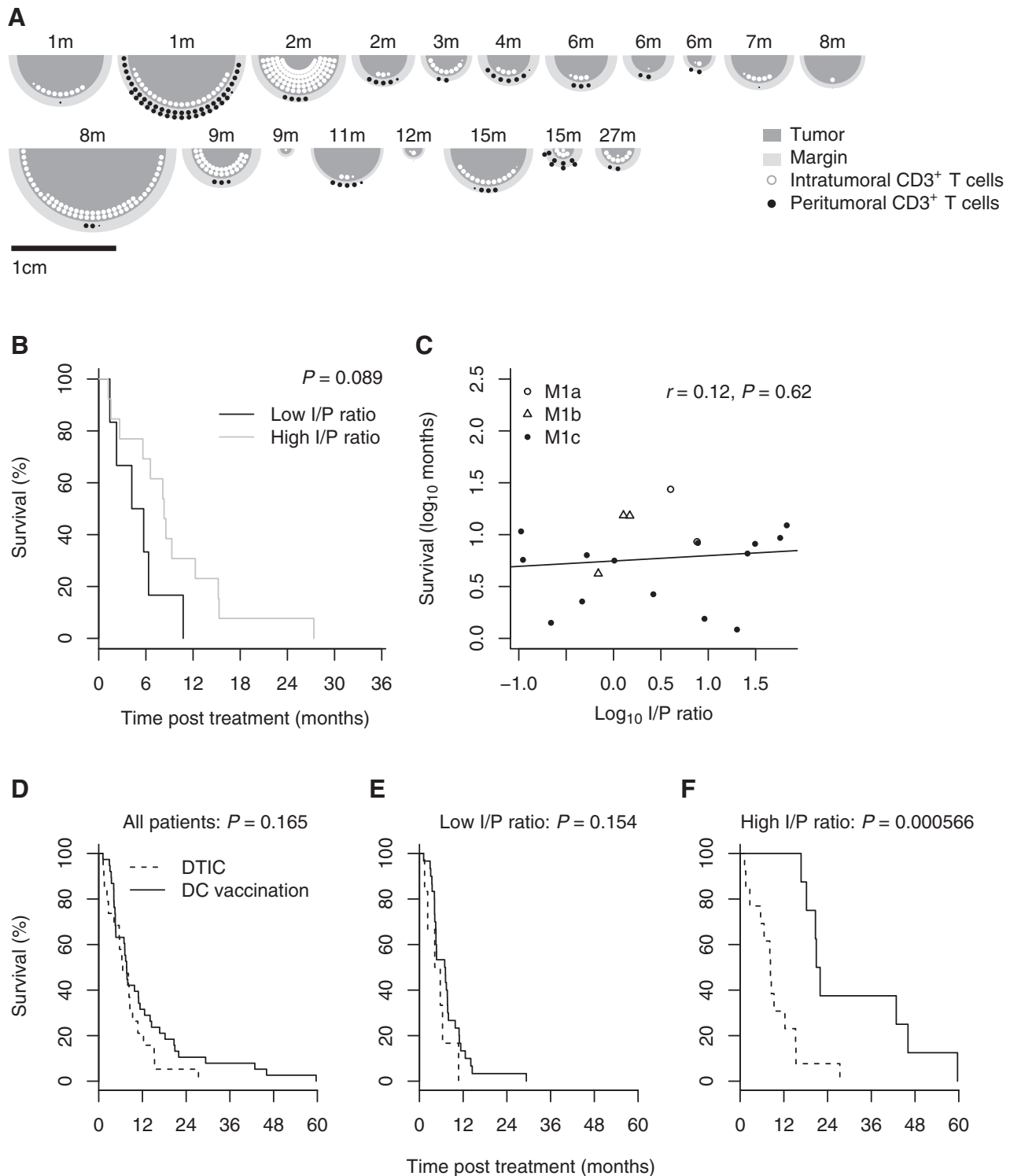
after the different treatments (Fig. 4E), indicating no survival benefit of DC-based immunotherapy when T-cell infiltration is weak. On the other hand, patients with high I/P ratios clearly survived longer after DC-based immunotherapy than patients with high I/P ratios treated with chemotherapy (Fig. 4F), and therefore seem to benefit from DC-based immunotherapy.

In summary, our matched analysis indicates that the I/P ratio could indeed be a predictive marker for DC-based immunotherapy: patients with high I/P ratios appear to benefit from receiving DC-based immunotherapy, whereas patients with low I/P ratios do not. It will be important to test this result in future randomized trials with DC vaccination and other immunotherapies.

Discussion

The rapidly evolving field of melanoma management and novel treatments mandates development of new biomarkers, especially given the high costs associated with current treatments and their substantial toxicity. While for targeted therapies, effective genetic tools are available to select patients who are most likely to benefit from treatment, that is, *BRAF* mutation status for vemurafenib, this is much less so for immunotherapy. Established factors that are known to correlate with survival after immunotherapy are mostly derived from metastases. When looking at immunotherapy in general, several studies have shown specific gene expression profiles, identified on pretreatment biopsies of melanoma metastases that correlate with outcome after immunotherapy (35). Besides gene profiling, multiple studies have focused on finding a predictive biomarker in the tumor microenvironment. For instance, the expression of FoxP3 and indoleamine 2,3-dioxygenase (IDO) in pretreatment biopsies of melanoma metastases have been linked to a positive association with clinical activity of anti-CTLA-4 antibody (36). Also, several studies investigated biomarkers for PD-1 pathway-blocking agents, in particular, the expression of the PD-1 ligand (PD-L1). Unfortunately, the many different PD-L1 clones and differences in assessment of the PD-L1 immunohistochemical staining complicate interpretation of the different studies (37). Nonetheless, in melanoma, there seems to be an association between PD-L1 expression on tumor cells and response to an anti-PD1-antibody (38). A recent study has focused both on the role of PD-L1 expression as well as CD8⁺ T cells in melanoma metastases and has shown that the density of pre-existing CD8⁺ T cells in both the tumoral area and the invasive tumor margin has a predictive value for the treatment outcome of patients receiving anti-PD-1 therapy (39). This study highlights the importance of pretreatment T-cell infiltration in the tumor area as it correlates with clinical outcome.

A disadvantage of most of these potential biomarkers is that tumor material from metastases can be difficult to access and only becomes available when patients reach systemic disease, and are therefore not suitable for decision-making at earlier stages of disease in which adjuvant immunotherapy might be beneficial. Furthermore, biomarkers observed in metastases might not be suitable for analysis of the primary tumor; for instance, the positive association between PD-L1 expression in metastases and melanoma-specific survival could not be confirmed in primary melanoma (40). Finally, markers like skin-infiltrating T cells isolated from DTH biopsies, although useful for monitoring

**Figure 4.**

Strong intratumoral T-cell infiltration is associated with a larger difference between DC vaccination and chemotherapy in a matched cohort. A, relative tumor size, size of peritumoral area, and CD3⁺ count of all patients in the chemotherapy cohort. Overall survival is shown above each tumor in months. Each large bullet point represents 1,000 T cells within the tumor (white) or in the peritumoral area (black). B, survival of patients treated with chemotherapy with low (<1, $n = 6$) and high (>1, $n = 13$) I/P ratios ($P = 0.089$). C, correlation between I/P ratio and survival in months are shown on a log-log scale for all patients. D, analysis of a combined cohort in which each of the 19 chemotherapy patients was matched to two patients from the DC vaccination cohorts (giving $n = 57$) that were matched on LDH and M1c substage. E, longer survival upon immunotherapy is not apparent in patients with low I/P ratio (<1; $n = 36$). F, yet, there is a significant difference for patients with high I/P ratio (>1; $n = 21$).

response to therapy, cannot be used for pretherapeutic decision-making.

In this study, we investigated whether the T-cell landscape within the primary tumor is relevant for survival of 77 patients with metastatic melanoma. Given partly contradicting results in the literature, our approach aimed to exploit a novel observer-independent methodology, and focused on patients who received immunotherapy as we expected the relevance of immune infiltration to be higher in this setting. Our results show that strong CD3⁺ T-cell infiltration into the primary tumor is an excellent early predictor of longer survival in metastatic melanoma patients receiving DC-based immunotherapy. This was revealed in a discovery cohort and confirmed in a validation cohort, which showed a clear correlation between intratumoral CD3⁺ T-cell infiltration and survival. Multivariate analyses showed that the I/P ratio improves upon and can be combined with LDH levels, measured at the time treatment decisions must be made. This led to the most accurate survival prediction in our validation cohort (AUC = 0.95), which also outperformed the conventional classification based on the TNM staging system.

T-cell recognition of tumor cells relies upon presentation of tumor-specific antigens on MHC molecules. Therefore, a potential mechanism explaining the relevance of T-cell infiltration might be that weak T-cell infiltration into the primary tumor reflects poor immunogenicity of the tumor and thus also of potential future metastases. Weak immunogenic tumors might also be more difficult to treat with immunotherapy. If so, then intratumoral T-cell infiltration should also be an appropriate marker for selecting patients for treatment with immunotherapy. Our analysis of the matched cohort provides support for this hypothesis. When data from randomized trials with DC vaccination become available, it will be possible to confirm this predictive biomarker status.

Consistently, with earlier studies, we found high intratumoral T-cell densities to be beneficial. However, in the cohorts studied here, a high T-cell density in the margin appears to be detrimental. In theory, a high marginal T-cell density could reflect an efficient homing rate of T cells from the circulation towards the tumor site. But it could also reflect a poor infiltration rate of T cells from the margin into the tumor. By measuring the I/P ratio, we focus on the infiltration rate, which predicts survival better than either of the individual densities. This might indicate that the infiltration rate is of particular importance in primary tumors, which may not be the case in metastases.

A major advantage of analyses of T-cell infiltration in primary tumors is that it can be assessed at initial diagnosis, which could select patients at high risk for metastatic disease and a high I/P ratio for adjuvant immunotherapy. This is indeed of interest, as we have recently shown that DC vaccination more frequently induced functional tumor-specific immune responses in stage III melanoma patients, compared with patients with metastatic disease, in which their presence correlated with clinical outcome (25, 41). Also, other studies have shown a benefit in terms of recurrence-free survival of treatment with the anti-CTLA-4 antibody in the adjuvant setting (42). Anti-CTLA-4 has recently been approved by the FDA for stage III melanoma patients; yet, it is still questionable whether anti-CTLA-4 is to be recommended as an adjuvant treatment because of its high toxicity, unknown late toxicities, and awaited data on overall survival. Identifying the right patients for adjuvant treatment (i.e., high risk patients who do not develop metastasis due to adjuvant treatment) would

optimize outcome, lower toxicity, and reduce costs. However, as we have not analyzed T-cell landscapes in stage III melanoma patients who did not develop distant metastases, further research is needed to determine the value of T-cell infiltration at this stage of disease.

Often, the immune system is not capable of efficiently controlling tumor growth despite proper infiltration of immune cells in the tumor. This inefficiency may be caused by several mechanisms, including decreased function of tumor antigen-specific T cells by immunosuppressive strategies caused by tumor cells. Therefore, we believe it will be important to reveal the phenotype and function of the CD3⁺ T cells. On the basis of previous studies examining the tumor microenvironment (8, 12, 43), we presume that the majority of the intratumoral cells are CD8⁺ T cells, as cytotoxic T cells are considered to be the most important effector cells in the tumor microenvironment. However, given that T cells in the margin appear detrimental, the peritumoral T cells could have a distinct, perhaps regulatory, phenotype. Further research should focus on unraveling the phenotype of the CD3⁺ T cells and the underlying mechanisms that give rise to the strong predictive value of the I/P ratio.

So far, most groups studying the correlation between TILs and patient survival classify the presence of TILs as absent, non-brisk and brisk (11, 15, 16). Some characterize TILs only as present or absent (44), while few use a more elaborate method that takes both the distribution and the density of TILs into account (12). Our method also takes distribution and density into account and does this in an automated and quantitative manner using the entire tumor slide. This way we are able to quantify all CD3⁺ T cells and assess their exact location. Computing I/P ratio requires sufficient availability of both peritumoral and intratumoral tissue. In this study, we aimed for including peritumoral tissue within 500–700 μ m around the tumor, which was possible in most cases. Thus, we believe that this method is practically applicable, provides highly accurate predictive information, and may lead to more reproducible results than manual qualitative assessment.

In summary, we have shown that T-cell infiltration into the tumor is a highly accurate predictor of survival in metastatic melanoma patients receiving DC vaccination: on average, patients with a high I/P ratio appear to benefit from DC-based immunotherapy, whereas patients with a low I/P ratio do not. The predictive power of the I/P ratio does not wane when taking into account established late-stage prognostic markers, which is remarkable given that DC vaccination was only administered at metastatic stage, years after resection of the primary tumor. This finding underscores the importance of the tumor microenvironment throughout the entire course of disease. From a clinical perspective, assessing the T-cell landscape at initial melanoma diagnosis is attractive for two reasons: primary tumors are more easily accessible than metastases, and the marker is available early on for diagnostic decision-making and, potentially, (adjuvant) treatment selection. As all immunotherapies are based on the same principle, this tool may be generally applicable for all types of immunotherapy. Further studies are warranted to confirm that strong intratumoral T-cell infiltration is indeed a predictive biomarker and to investigate the prognostic and predictive potentials of the T-cell landscape for other types of immunotherapy in melanoma. Finally, this approach may be useful for the investigation of other tumor types, especially for other immunogenic tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Vasaturo, K.F. Bol, C.J.A. Punt, J.H.J.M. van Krieken, J. Textor, I.J.M. de Vries, C.G. Figdor

Development of methodology: A. Vasaturo, C.J.A. Punt, J.H.J.M. van Krieken, J. Textor, I.J.M. de Vries, C.G. Figdor

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Vasaturo, A. Halilovic, K.F. Bol, D.I. Verweij, P.J.T.A. Groenen, I.J.M. de Vries

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Vasaturo, A. Halilovic, K.F. Bol, D.I. Verweij, W.A.M. Blokx, C.J.A. Punt, P.J.T.A. Groenen, J.H.J.M. van Krieken, J. Textor, I.J.M. de Vries, C.G. Figdor

Writing, review, and/or revision of the manuscript: A. Vasaturo, A. Halilovic, K.F. Bol, D.I. Verweij, W.A.M. Blokx, C.J.A. Punt, J.H.J.M. van Krieken, J. Textor, I.J.M. de Vries, C.G. Figdor

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Vasaturo, A. Halilovic, K.F. Bol, D.I. Verweij, P.J.T.A. Groenen

Study supervision: C.J.A. Punt, I.J.M. de Vries, C.G. Figdor

Acknowledgments

The authors thank the technicians involved in the DC vaccination studies: Nicole Scharenborg, Annemiek de Boer, Mandy van de Rakt, Michel Olde Nordkamp, Jeanette Pots, Tom van Oorschot, and Tjitske Duiveman-de Boer and the technicians of the IHC Laboratory, under the supervision of Monique Link. The authors also thank Peter van Zwam, Jeroen van de Laak, and Harm Westdorp for their efforts in the initial phase of this research.

Grant Support

This work was supported by KWO grant KUN2009-4402 from the Dutch Cancer Society. I.J.M. de Vries received a NWO Vici grant (918.14.655). C.G. Figdor received the NWO Spinoza award and ERC Advanced grant PATHFINDER (269019). J. Textor was supported by a NWO Earth and Life Sciences grant (823.02.014).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 30, 2015; revised March 7, 2016; accepted March 29, 2016; published OnlineFirst April 11, 2016.

References

- Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953-2008—are recent generations at higher or lower risk? *Int J Cancer* 2013; 132:385-400.
- Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol* 2009;27:6199-206.
- Palathinkal DM, Sharma TR, Koon HB, Bordeaux JS. Current systemic therapies for melanoma. *Dermatol Surg* 2014;40:948-63.
- Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell* 2015;161: 205-14.
- Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, van den Eertwegh AJ, et al. Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. *Cancer Immunol Immunother* 2014;63:449-58.
- Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012; 12:298-306.
- Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 2011;331:1565-70.
- Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Page C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006;313:1960-4.
- Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani C, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med* 2003;348:203-13.
- Liu H, Zhang T, Ye J, Li H, Huang J, Li X, et al. Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small cell lung cancer. *Cancer Immunol Immunother* 2012;61:1849-56.
- Clark WH Jr, Elder DE, Guerry Dt, Braitman LE, Trock BJ, Schultz D, et al. Model predicting survival in stage I melanoma based on tumor progression. *J Natl Cancer Inst* 1989;81:1893-904.
- Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol* 2012;30:2678-83.
- Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer* 1996;77:1303-10.
- Bogunovic D, O'Neill DW, Belitskaya-Levy I, Vacic V, Yu YL, Adams S, et al. Immune profile and mitotic index of metastatic melanoma lesions enhance clinical staging in predicting patient survival. *Proc Natl Acad Sci U S A* 2009;106:20429-34.
- Taylor RC, Patel A, Panageas KS, Busam KJ, Brady MS. Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. *J Clin Oncol* 2007;25:869-75.
- Mandala M, Imberti GL, Piazzalunga D, Belfiglio M, Labianca R, Barberis M, et al. Clinical and histopathological risk factors to predict sentinel lymph node positivity, disease-free and overall survival in clinical stages I-II AJCC skin melanoma: outcome analysis from a single-institution prospectively collected database. *Eur J Cancer* 2009;45:2537-45.
- Burton AL, Roach BA, Mays MP, Chen AF, Ginter BA, Vierling AM, et al. Prognostic significance of tumor infiltrating lymphocytes in melanoma. *Am Surg* 2011;77:188-92.
- Karagiannis P, Fittall M, Karagiannis SN. Evaluating biomarkers in melanoma. *Front Oncol* 2014;4:383.
- Lesterhuis WJ, Aarntzen EH, De Vries IJ, Schuurhuis DH, Figdor CG, Adema CJ, et al. Dendritic cell vaccines in melanoma: from promise to proof? *Crit Rev Oncol Hematol* 2008;66:118-34.
- Jacobs JF, Punt CJ, Lesterhuis WJ, Suttmuller RP, Brouwer HM, Scharenborg NM, et al. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. *Clin Cancer Res* 2010;16:5067-78.
- Tel J, Aarntzen EH, Baba T, Schreiber G, Schulte BM, Benitez-Ribas D, et al. Natural human plasmacytoid dendritic cells induce antigen-specific T-cell responses in melanoma patients. *Cancer Res* 2013;73:1063-75.
- Wilgenhof S, Van Nuffel AM, Benteyn D, Corthals J, Aerts C, Heirman C, et al. A phase IB study on intravenous synthetic mRNA electroporated dendritic cell immunotherapy in pretreated advanced melanoma patients. *Ann Oncol* 2013;24:2686-93.
- Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical use of dendritic cells for cancer therapy. *Lancet Oncol* 2014;15:e257-67.
- de Vries IJ, Bernsen MR, Lesterhuis WJ, Scharenborg NM, Strijk SP, Gerritsen MJ, et al. Immunomonitoring tumor-specific T cells in delayed-type hypersensitivity skin biopsies after dendritic cell vaccination correlates with clinical outcome. *J Clin Oncol* 2005;23:5779-87.
- Aarntzen EH, Bol K, Schreiber G, Jacobs JF, Lesterhuis WJ, van Rossum MM, et al. Skin-test infiltrating lymphocytes early predict clinical outcome of dendritic cell based vaccination in metastatic melanoma. *Cancer Res* 2012;72:6102-10.
- Wimmers F, Aarntzen EH, Schreiber G, Jacobs JF, Ja Punt C, Figdor CG, et al. Early predictive value of multifunctional skin-infiltrating lymphocytes in anticancer immunotherapy. *Oncoimmunology* 2014;3:e27219.
- Lesterhuis WJ, de Vries IJ, Schreiber G, Lambeck AJ, Aarntzen EH, Jacobs JF, et al. Route of administration modulates the induction of dendritic cell

Vasaturo et al.

- vaccine-induced antigen-specific T cells in advanced melanoma patients. *Clin Cancer Res* 2011;17:5725–35.
28. Lesterhuis WJ, Schreibelt G, Scharenborg NM, Brouwer HM, Gerritsen MJ, Croockewit S, et al. Wild-type and modified gp100 peptide-pulsed dendritic cell vaccination of advanced melanoma patients can lead to long-term clinical responses independent of the peptide used. *Cancer Immunol Immunother* 2011;60:249–60.
 29. Aarntzen EH, Schreibelt G, Bol K, Lesterhuis WJ, Croockewit AJ, de Wilt JH, et al. Vaccination with mRNA-electroporated dendritic cells induces robust tumor antigen-specific CD4+ and CD8+ T cells responses in stage III and IV melanoma patients. *Clin Cancer Res* 2012;18:5460–70.
 30. Aarntzen EH, De Vries JJ, Lesterhuis WJ, Schuurhuis D, Jacobs JF, Bol K, et al. Targeting CD4(+) T-helper cells improves the induction of antitumor responses in dendritic cell-based vaccination. *Cancer Res* 2013;73:19–29.
 31. Bol KF, Figdor CG, Aarntzen EH, Welzen ME, van Rossum MM, Blokx WA, et al. Intranodal vaccination with mRNA-optimized dendritic cells in metastatic melanoma patients. *Oncoimmunology* 2015;4:e1019197.
 32. Mansfield JR. Cellular context in epigenetics: quantitative multicolor imaging and automated per-cell analysis of miRNAs and their putative targets. *Methods* 2010;52:271–80.
 33. Stack EC, Wang C, Roman KA, Hoyt CC. Multiplexed immunohistochemistry, imaging, and quantitation: a review, with an assessment of Tyramide signal amplification, multispectral imaging and multiplex analysis. *Methods* 2014;70:46–58.
 34. Hansen BB, Klopfer SO. Optimal full matching and related designs via network flows. *J Comput Graph Stat* 2006;15:609–27.
 35. Gajewski TF, Louahed J, Brichard VG. Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy. *Cancer J* 2010;16:399–403.
 36. Hamid O, Schmidt H, Nissan A, Ridolfi L, Aamdal S, Hansson J, et al. A prospective phase II trial exploring the association between tumor micro-environment biomarkers and clinical activity of ipilimumab in advanced melanoma. *J Transl Med* 2011;9:204.
 37. Mahoney KM, Atkins MB. Prognostic and predictive markers for the new immunotherapies. *Oncology* 2014;28Suppl 3:39–48.
 38. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, et al. Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. *Clin Cancer Res* 2014;20:5064–74.
 39. Tumeo PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
 40. Madore J, Vilain RE, Menzies AM, Kakavand H, Wilmott JS, Hyman J, et al. PD-L1 expression in melanoma shows marked heterogeneity within and between patients: implications for anti-PD-1/PD-L1 clinical trials. *Pigment Cell Melanoma Res* 2015;28:245–53.
 41. Bol KF, Aarntzen EH, Hout FE, Schreibelt G, Creemers JH, Lesterhuis WJ, et al. Favorable overall survival in stage III melanoma patients after adjuvant dendritic cell vaccination. *Oncoimmunology* 2015;5:e1057673.
 42. Eggermont AM, Chiarion-Sileni V, Grob JJ, Dummer R, Wolchok JD, Schmidt H, et al. Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORTC 18071): a randomised, double-blind, phase 3 trial. *Lancet Oncol* 2015;16:522–30.
 43. Tjin EP, Krebbers G, Meijlink KJ, van de Kastele W, Rosenberg EH, Sanders J, et al. Immune-escape markers in relation to clinical outcome of advanced melanoma patients following immunotherapy. *Cancer Immunol Res* 2014;2:538–46.
 44. de Moll EH, Fu Y, Qian Y, Perkins SH, Wieder S, Gnjatic S, et al. Immune biomarkers are more accurate in prediction of survival in ulcerated than in non-ulcerated primary melanomas. *Cancer Immunol Immunother* 2015;64:1193–203.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

T-cell Landscape in a Primary Melanoma Predicts the Survival of Patients with Metastatic Disease after Their Treatment with Dendritic Cell Vaccines

Angela Vasaturo, Altuna Halilovic, Kalijn F. Bol, et al.

Cancer Res 2016;76:3496-3506. Published OnlineFirst April 11, 2016.

Updated version Access the most recent version of this article at:
doi:[10.1158/0008-5472.CAN-15-3211](https://doi.org/10.1158/0008-5472.CAN-15-3211)

Supplementary Material Access the most recent supplemental material at:
<http://cancerres.aacrjournals.org/content/suppl/2016/04/09/0008-5472.CAN-15-3211.DC1>

Cited articles This article cites 44 articles, 15 of which you can access for free at:
<http://cancerres.aacrjournals.org/content/76/12/3496.full#ref-list-1>

Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at:
<http://cancerres.aacrjournals.org/content/76/12/3496.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cancerres.aacrjournals.org/content/76/12/3496>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.