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T cell responses against tumor associated antigens and prognosis in colorectal cancer patients

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Abstract

Introduction: Spontaneous T cell responses against specific tumor-associated antigens (TAA) are frequently detected in peripheral blood of tumor patients of various histiotypes. However, little is known about whether these circulating, spontaneously occurring, TAA-reactive T cells influence the clinical course of disease.

Methods: Fifty-four HLA-A2 positive colorectal cancer patients had been analyzed for the presence of T cell responses against epitopes derived from the TAA Ep-CAM, her-2/neu, and CEA either by ELISPOT assay or by intracellular cytokine staining. Then, Kaplan-Meier survival analysis was performed comparing T-cell-responders and T-cell-non-responders. For comparison, a group of T-cell-non-responders was compiled stringently matched to T-cell-responders based on clinical criteria and also analyzed for survival.

Results: Sixteen out of 54 patients had a detectable T cell response against at least one of the three tested TAA. Two out of 21 patients (9.5%) with limited stage of disease (UICC I and II) and 14 out of 33 patients (42.4%) with advanced disease (UICC III and IV) were T cell response positive. Comparing all T-cell-responders (n = 16) and all T-cell-non-responders (n = 38), no survival difference was found. In an attempt to reduce the influence of confounding clinical factors, we then compared 16 responders and 16 non-responders in a matched group survival analysis; and again no survival difference was found (p = 0.7).

Conclusion: In summary, we found no evidence that spontaneous peripheral T cell responses against HLA-A2-binding epitopes of CEA, her-2/neu and Ep-CAM are a strong prognostic factor for survival.

Introduction

The importance of the immune system in containing tumor growth is supported by animal studies and various observations in humans [1,2]. These include increased prevalence of certain tumors following immunosuppression as well as the demonstration, that the presence of intralesional T cells is correlated with improved clinical outcome in various solid tumors [1,3-6]. In particular in CRC, the presence of CD8+ T cells within the tumor microenvironment was significantly associated with a better survival in several studies [3,7-9]. However, the antigen-specificity of these cells was not determined. T cell responses against specific tumor-associated antigens (TAA) are frequently detected in the peripheral blood of tumor patients [reviewed in [10]] of various histiotypes including colorectal cancer [11], melanoma [12,13], acute myeloid leukemia [14], breast cancer [15], neuroblastoma [16], and head and neck cancer [17]. Data from selected single patients suggest a favorable clinical course in patients with peripheral, spontaneous TAA-directed T cells [18,19]. However, this type of analysis does not allow firm conclusions. The only study comparing clinical outcome of patients with presence of antigen-specific immune responses including natural as well as vaccine-induced antibodies against melanoma antigen GM2 showed an improved survival in favor of immune responders [20]. TAA-directed T cell responses can reliably be induced using various vaccination approaches [reviewed in [21]]. Several recent reports have found a correlation between induction of a TAA-directed T cell response by vaccination and clinical response [22-25]. Preliminary data also suggest a possibly favorable clinical effect of vaccine-induced T cells in adjuvant vaccination [26-29]. Taken together, these data lead to the question, whether the presence of spontaneous TAA-specific T cells might be a positive prognostic factor. So far, however, no study has systematically compared survival data of patients with and without presence of a spontaneous TAA-directed T cell response.

In previous studies, we have demonstrated spontaneous T cell responses against the TAA CEA, Ep-CAM, or her-2/neu in peripheral blood of approximately 25% of colorectal cancer patients [11,30]. These cells were identified in functional T cell assays by antigen-induced IFN γ production. More detailed analyses in some samples revealed a CD3+ CD8+ IFN γ + CD69+ CD45RA+ phenotype [11], indicative of an effector T cell subset that is able to directly mediate tumor cell lysis [19]. Spontaneous TAA-specific T cells with the potential of effector cells should, theoretically, be capable of destroying tumor cells and thereby lead to elimination of residual disease or prevent tumor progression. To investigate whether a peripheral, spontaneous T cell response has an effect on the clinical outcome of tumor patients, we analyzed survival data of CRC

patients with a TAA-directed T cell response and compared these data with the clinical course of CRC patients without detectable T cell response.

Patients, materials, and methods

Patient selection and T cell assays

After institutional review board approval and informed consent, peripheral blood mononuclear cells from 132 patients with CRC in all stages of disease had been prospectively collected and frozen for T cell analysis. All analyses have been performed in compliance with the Helsinki Declaration. Fifty-four patients were tested positive for HLA-A2 and were subsequently analyzed for the presence of T cell responses against the HLA-A*0201 presented T cell epitopes Ep-CAM p263-271 [31], her-2/neu p654-662 [32,33], and CEA p571-579 [34] either by ELISPOT assay or by intracellular cytokine staining. HLA analysis, ELISPOT, and intracellular cytokine staining were performed as previously described [11,30]. Positive responses were defined as previously described [11,30].

Survival analysis

First, we performed a Kaplan-Meier survival analysis comparing all T-cell-responders and all T-cell-non-responders. Additionally, a two-sided log rank test was used to test statistical significance. Then, in an attempt to reduce the influence of external factors, we compiled a patient group from non-responders matching them to responders according to the following criteria: UICC stage of disease, gender, presence of clinically detectable tumor at time of blood draw, duration of disease until blood draw, age at first diagnosis, and previous therapy. Survival in both groups was compared using Kaplan-Meier analysis and by a two-sided log rank test. A level of $p < 0.05$ was considered significant. SPSS (11.5) software was used.

Results

Patients, T cell response, survival of responders and non-responders

Fifty-four HLA-A2 positive CRC patients who had been analyzed for T cell responses were included in this study [11,30] and retrospectively analyzed for survival. The overall survival rate was 66.7% at a median of 27.5 months follow-up after blood draw for T cell analysis. In 16 out of 54 patients a total number of 26 T cell responses (between 10 and 1110 specific T cell per 10^6 PBMC) against one of the three TAA Ep-CAM, her-2/neu, and CEA had been detected. Comparing all responders ($n = 16$) and all non-responders ($n = 38$), no survival difference was found in a two-sided log rank test ($p = 0.4$; Fig. 1A). A very slight trend toward better survival was found in favor of non-responders, which is, however, far from being statistically significant. Since we have previously observed that T cell responses occur more frequently in patients with advanced stages of disease, we compared clinical

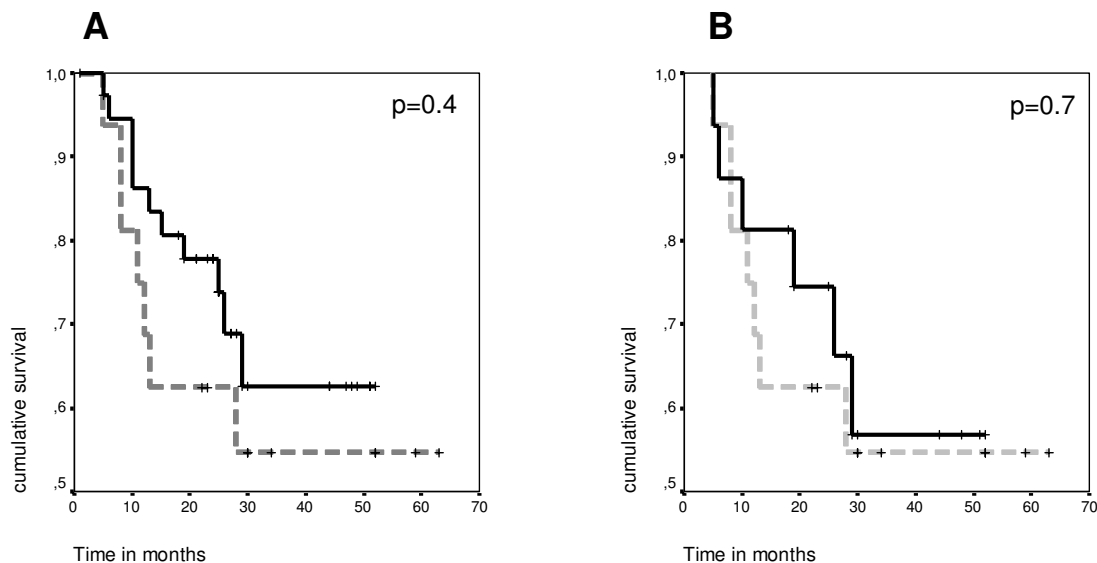


Figure 1

Kaplan-Meier survival analyses of colorectal cancer patients based on their T cell response state. Dashed lines refer to T-cell-responders, full lines refer to T-cell-non-responders, crosses mark censored cases. Time point 0 refers to the time of blood draw for T cell analysis. **A.** Two groups of CRC patients (total $n = 54$) were analyzed for survival after T cell analysis. One group had a spontaneous T cell response against the tumor associated antigens CEA, Ep-CAM, or her-2/neu ($n = 16$, dashed line). The other group had no T cell response against these antigens ($n = 38$, full line). No survival difference between the groups was found (log rank, $p = 0.4$). **B.** Two matched groups of CRC patients (total $n = 32$) were analyzed for survival after T cell analysis. One group had a spontaneous T cell response against tumor antigens CEA, Ep-CAM, or her-2/neu ($n = 16$, dashed line). The other group had no T cell response against these antigens and was selected by having similar clinical characteristics for stage, gender, presence of clinically detectable tumor at time of blood draw, duration of disease, age, and prior therapy ($n = 16$, full line). No survival difference was found (log rank, $p = 0.7$).

data for all patients; and found that among non-responders only 50% (19 out of 38) had clinical stage III or IV disease while 87.5% (14 out of 16) responding patients had stage III or IV disease. Thus, the data on survival are possibly based on a strong stage-related bias. Therefore, we subsequently used an approach matching non-responders to responders.

Survival in matched patient groups

Sixteen patients from the above group of 38 non-responders were matched with 16 responders to obtain two groups comparable for potentially confounding clinical factors, in particular stage of disease (see table 1). At the time of the survival analysis 13 of the total of 32 (40.6%) patients had died: seven T-cell-responders ($n = 1$ stage III,

Table 1: Patient characteristics

Peripheral spontaneous T cell response against TAA	Positive	Negative (matched)	Negative (total)
Number of patients	16	16	38
Age at first diagnosis, mean \pm SD	61.2 \pm 12.1	59.9 \pm 9.1	60.8 \pm 11.1
Male/Female	11/5	11/5	19/19
Duration of disease until T cell analysis (mean)	31.8	30.2	27.4
Stage (UICC)			
I	0	0	1 (2.6%)
II	2 (12.5%)	2 (12.5%)	18 (47.4%)
III	3 (18.8%)	3 (18.8%)	5 (13.2%)
IV	11 (68.8%)	11 (68.8%)	14 (36.8%)
Previous therapy			
CT	9 (56.3%)	12 (75%)	18 (47.4%)
RT	3 (18.8%)	4 (25%)	7 (18.4%)
Surgery	12 (75%)	14 (87.5%)	30 (78.9%)
Patients without evidence of tumor at time of blood draw	4 (25%, 2 II, 2 III)	4 (25%, 2 II, 2 III)	17 (45%, 13 II, 3 III, 1 IV)
Time from blood draw until death or last contact, mean \pm SD	28.1 \pm 19.2	27.4 \pm 15.0	25.7 \pm 14.0

Abbreviations: SD standard deviation, CT chemotherapy (5-FU + folic acid, in few cases plus Irinotecan), RT radiotherapy

n = 6 stage IV) and six T-cell-non-responders (n = 1 stage III, n = 5 stage IV). All deaths were CRC-related. Median time to death among T-cell-responders was 11 months, among T-cell-non-responders 14.5 months. The calculated mean survival time after blood draw for patients without T cell response was 37.0 months (\pm 4.8 SEM) with a 95% confidence interval 27.5–46.5. Mean survival of T-cell-responders was 40.2 months (\pm 6.5 SEM) with 95% confidence interval of 27.5–52.9 (Fig. 1B). In a two-sided log rank test, survival did not show a statistically significant difference between responders and non-responders ($p = 0.7$). Of note, one to two years after blood draw, patients without T cell response had an up to 20% higher survival rate (approx. 80% vs. approx 60%). These results were, however, not significant. In a two-sided test with $\beta = 0.2$, a survival difference of 70% could have been considered significant at a level of $\alpha = 0.05$ in a population of this size.

Discussion

In the present study, we analyzed the clinical course of colorectal cancer patients with or without T cells reactive against HLA-A2-binding epitopes of Ep-Cam, her-2/neu, and CEA. No survival difference between T-cell-responders and T-cell-non-responders was found. This result has to be interpreted cautiously due to the small number of HLA-A2+ patients responding to the above antigens. Obviously, these small numbers cause a high beta error. Thus, this study is only a first indication that the tested spontaneous T cell responses are not important prognostic factors for survival.

The second limitation of our study is that the known repertoire of TAA as potentially important T cell targets in

CRC grows every year; and our T cell analysis included only a fraction of potential epitopes. Various other CRC-associated antigens, such as MUC1 or p53, and additional MHC class I antigenic epitopes of CEA and her2-neu have been described [summarized in [35]]. T cell responses against antigens in addition to the ones tested here could potentially play a role in immune surveillance of CRC.

Immune surveillance is understood as a complex process in which T cells and tumor cells influence each other in several ways ["immunoediting", [1]]. There are various potential factors related to tumor cells as well as T cells which may explain a lack of survival effect by TAA-specific T cells. The frequency of TAA-specific T cells detected in most patients was quite low in the range of 10 to 100 T cells per 10^6 PBMC. These numbers may be too low to control tumor growth especially in patients with a higher tumor burden. Furthermore, a general T cell dysfunction including anergic T cells and T cells with downregulated CD3-zeta chains has been described in CRC patients [36,37]. It is possible that the specific T cells detected in the present study are functionally unable to destroy tumor cells. This assumption, however, is not supported by our previous finding that TAA-specific T cells have an effector potential as analyzed in selected patients [11]. Since we have analyzed peripheral blood, we do not know if the circulating T cells have the potential to migrate to the tumor site or compartments where CRC cells frequently migrate to including lymph node, liver and bone marrow.

Furthermore, tumor cells may not be recognizable by TAA-specific T cells. It has been shown that CRC cell lines secrete immunosuppressive cytokines and that development of T cell responses is impeded due to low HLA

expression and lack of intercellular adhesion molecule-1 (ICAM-1) and HLA-DR [38,39]. This is especially relevant considering the fact that TAA-specific T cell responses in peripheral blood are more frequently detectable in advanced stages of CRC [11,40], as well as other tumors [40,41]. These data led to the hypothesis that metastasizing of tumor cells to lymph nodes is a prerequisite for the development of circulating T cell responses [11,42]. Furthermore, the presence of TAA-directed T cell responses may have selected immune escape tumor variants. A broad variety of tumor escape mechanisms, such as antigen loss or loss of HLA expression, is described in various clinical conditions [43]. It is possible that we encounter similar mechanisms in the present study since malignant cells had grown *in vivo* during the presence of specific T cell responses. Finally, the role of suppressor and regulatory T cells in this specific context is unknown.

Taken together, no evidence was found that peripheral, spontaneous T cell responses against HLA-A*0201-binding epitopes of CEA, Ep-CAM, or her-2/neu influence survival of CRC patients. Since the low patient number limits the conclusion, further studies should investigate more patients, more detailed function and migratory pattern of spontaneous T cell responses as well as the genetic profile of the tumor; and consider a broader antigen and epitope repertoire. These studies could have implications for vaccination therapy as we learn more about why immune surveillance may fail to control tumors and if the presence of a natural T cell response may impact on the efficacy of a vaccine.

Abbreviations

CEA carcinoembryonic antigen, CRC, colorectal carcinoma; ELISPOT, enzyme-linked immunospot; IFN γ , Interferon- γ ; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cells, TAA, tumor associated antigen.

Competing interests

The author(s) declare that they have no competing interests.

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References

- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD: **Cancer immunoeediting: from immunosurveillance to tumor escape.** *Nat Immunol* 2002, **3**:991-8.
- Boon T, van Baren N: **Immunosurveillance against cancer and immunotherapy – synergy or antagonism?** *N Engl J Med* 2003, **348**:252-4.
- Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H, Ohtani H: **CD8+ T cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer.** *Cancer Res* 1998, **58**:3491-3494.
- Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G: **Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer.** *N Engl J Med* 2003, **348**:203-213.
- Mihm M, Clemente C, Cascinelli N: **Tumor infiltrating lymphocytes in lymph node melanoma metastases: a histopathologic prognostic indicator and an expression of local immune response.** *Lab Invest* 1996, **74**:43-47.
- Schumacher K, Haensch W, Roefzaad C, Schlag PM: **Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas.** *Cancer Res* 2001, **61**:3932-3936.
- Ropponen KM, Eskelinen MJ, Lippinen PK, Alhava E, Kosma VM: **Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer.** *J Pathol* 1997, **182**:318-24.
- Oberg A, Samii S, Stenling R, Lindmark G: **Different occurrence of CD8+, CD45R0+, and CD68+ immune cells in regional lymph node metastases from colorectal cancer as potential prognostic predictors.** *Int J Colorectal Dis* 2002, **17**:25-9.
- Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE: **Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer.** *Oncol Rep* 2003, **10**:309-13.
- Nagorsen D, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U: **Natural T cell immunity against cancer.** *Clin Cancer Res* 2003, **9**:4296-303.
- Nagorsen D, Keilholz U, Rivoltini L, Schmittel A, Letsch A, Asemissen AM, Berger G, Buhr HJ, Thiel E, Scheibenbogen C: **Natural T cell response against MHC class I epitopes of epithelial cell adhesion molecule, her-2/neu, and carcinoembryonic antigen in patients with colorectal cancer.** *Cancer Res* 2000, **60**:4850-4854.
- Pittet MJ, Valmori D, Dunbar PR, Speiser DE, Lienard D, Lejeune F, Fleischhauer K, Cerundolo V, Cerottini JC, Romero P: **High frequencies of naive Melan-A/MART-1-specific CD8(+) T cells in a large proportion of human histocompatibility leukocyte antigen (HLA)-A2 individuals.** *J Exp Med* 1999, **190**:705-715.
- Lee PP, Yee C, Savage PA, Fong L, Brockstedt D, Weber JS, Johnson D, Swetter S, Thompson J, Greenberg PD, Roederer M, Davis MM: **Characterization of circulating T cells specific for tumor-associated antigens in melanoma patients.** *Nat Med* 1999, **5**:677-685.
- Scheibenbogen C, Letsch A, Thiel E, Schmittel A, Mailaender V, Baerwolf S, Nagorsen D, Keilholz U: **CD8 T-cell responses to Wilms tumor gene product WT1 and proteinase 3 in patients with acute myeloid leukemia.** *Blood* 2002, **100**:2132-2137.
- Feuerer M, Beckhove P, Bai L, Solomayer EF, Bastert G, Diel IJ, Pedain C, Oberriederer M, Schirrmacher V, Umansky V: **Therapy of human tumors in NOD/SCID mice with patient-derived reactivated memory T cells from bone marrow.** *Nat Med* 2001, **7**:452-458.
- Rodolfo M, Luksch R, Stockert E, Chen YT, Collini P, Ranzani T, Lombardo C, Dalerba P, Rivoltini L, Arienti F, Fossati-Bellani F, Old LJ, Parmiani G, Castelli C: **Antigen-specific immunity in neuroblastoma patients: antibody and T-cell recognition of NY-ESO-1 tumor antigen.** *Cancer Res* 2003, **63**:6948-55.
- Albers A, Ferris R, Whiteside T, DeLeo A: **Immune Responses to P53 in Patients with Cancer: Elevated Frequencies of Tetramer+ P53 Peptide-Specific T Cells and Regulatory CD4+CD25+ Cells at Tumor Sites Compared to the Peripheral Circulation.** *J Immunother* 2003, **26**(6): Abstract
- Karanikas V, Colau D, Baurain JF, Chiari R, Thonnard J, Gutierrez-Roelens I, Goffinet C, Van Schaftingen EV, Weynants P, Boon T, Coulie PG: **High frequency of cytolytic T lymphocytes directed against a tumor-specific mutated antigen detectable with HLA tetramers in the blood of a lung carcinoma patient with long survival.** *Cancer Res* 2001, **61**:3718-3724.
- Valmori D, Scheibenbogen C, Dutoit V, Nagorsen D, Asemissen AM, Rubio-Godoy V, Rimoldi D, Guillaume P, Romero P, Schadendorf D, Lipp M, Dietrich PY, Thiel E, Cerottini JC, Lienard D, Keilholz U: **Circulating tumor-reactive CD8(+) T cells in melanoma patients contain a CD45RA(+)CCR7(-) effector subset exert-**

- ing *ex vivo* tumor-specific cytolytic activity. *Cancer Res* 2002, **62**:1743-1750.
20. Livingston PO, Wong GY, Adluri S, Tao Y, Padavan M, Parente R, Hanlon C, Calves MJ, Helling F, Ritter G, Oetten HF, Lloyd JO: **Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside.** *J Clin Oncol* 1994, **12**:1036-44.
 21. Scheibenbogen C, Letsch A, Schmittel A, Asemissen AM, Thiel E, Keilholz U: **Rational peptide-based tumour vaccine development and T cell monitoring.** *Seminars in Cancer biology* 2003, **13**:423-429.
 22. Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, Pineiro L, Steinman R, Fay J: **Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine.** *Cancer Res* 2001, **61**:6451-6458.
 23. Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM, Engleman EG: **Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy.** *Proc Natl Acad Sci U S A* 2001, **98**:8809-14.
 24. Coulie PG, Karanikas V, Colau D, Lurquin C, Landry C, Marchand M, Dorval T, Brichard V, Boon T: **A monoclonal cytolytic T-lymphocyte response observed in a melanoma patient vaccinated with a tumor-specific antigenic peptide encoded by gene MAGE-3.** *Proc Natl Acad Sci U S A* 2001, **98**:10290-5.
 25. Belli F, Testori A, Rivoltini L, Maio M, Andreola G, Sertoli MR, Gallino G, Piris A, Cattelan A, Lazzari I, Carrabba M, Scita G, Santantonio C, Pilla L, Tragni G, Lombardo C, Arienti F, Marchiano A, Queirolo P, Bertolini F, Cova A, Lamaj E, Ascani L, Camerini R, Corsi M, Cascinelli N, Lewis JJ, Srivastava P, Parmiani G: **Vaccination of metastatic melanoma patients with autologous tumor-derived heat shock protein gp96-peptide complexes: clinical and immunologic findings.** *J Clin Oncol* 2002, **20**:4169-4180.
 26. Wang F, Bade E, Kuniyoshi C, Spears L, Jeffery G, Marty V, Groshen S, Weber J: **Phase I trial of a MART-1 peptide vaccine with incomplete Freund's adjuvant for resected high-risk melanoma.** *Clin Cancer Res* 1999, **5**:2756-65.
 27. Slingluff CL Jr, Yamshchikov G, Neese P, Galavotti H, Eastham S, Engelhard VH, Kittlesen D, Deacon D, Hibbits S, Grosh WW, Petroni G, Cohen R, Wiernasz C, Patterson JW, Conway BP, Ross WG: **Phase I trial of a melanoma vaccine with gp100(280-288) peptide and tetanus helper peptide in adjuvant: immunologic and clinical outcomes.** *Clin Cancer Res* 2001, **7**:3012-24.
 28. Lee P, Wang F, Kuniyoshi J, Rubio V, Stuges T, Groshen S, Gee C, Lau R, Jeffery G, Margolin K, Marty V, Weber J: **Effects of interleukin-12 on the immune response to a multi-peptide vaccine for resected metastatic melanoma.** *J Clin Oncol* 2001, **19**:3836-47.
 29. Weber J, Sondak VK, Scotland R, Phillip R, Wang F, Rubio V, Stuge TB, Groshen SG, Gee C, Jeffery GG, Sian S, Lee PP: **Granulocyte-macrophage-colony-stimulating factor added to a multi-peptide vaccine for resected Stage II melanoma.** *Cancer* 2003, **97**:186-200.
 30. Nagorsen D, Scheibenbogen C, Schaller G, Leigh B, Schmittel A, Letsch A, Thiel E, Keilholz U: **Differences in T cell immunity towards tumor associated antigens between colorectal cancer and breast cancer.** *Int J Cancer* 2003, **105**:221-225.
 31. Ras E, Burg SH, van der, Zegveld ST, Brandt RMP, Kuppen PJK, Offringa R, Warnarr SO, Velde CJH, van de, Melief CJ: **Identification of potential HLA-A 0201 restricted CTL epitopes derived from the epithelial cell adhesion molecule (Ep-CAM) and the carcinoembryonic antigen (CEA).** *Human Immunology* 1997, **53**:81-89.
 32. Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ: **Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER-2/neu-derived peptide.** *Proc Natl Acad Sci U S A* 1995, **92**:432-436.
 33. Peiper M, Goedegebuure PS, Linehan DC, Ganguly E, Douville CC, Eberlein TJ: **The Her-2/neu-derived peptide p654-662 is a tumor-associated antigen in human pancreatic cancer recognized by cytotoxic T lymphocytes.** *Eur J Immunol* 1997, **27**:1115-1123.
 34. Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J: **Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine.** *J Natl Cancer Inst* 1995, **87**:982-990.
 35. Titu LV, Monson JR, Greenman J: **The role of CD8(+) T cells in immune responses to colorectal cancer.** *Cancer Immunol Immunother* 2002, **51**:235-47.
 36. Pellegrini P, Berghella AM, Del Beato T, Cicia S, Adorno D, Casciani CU: **Disregulation in TH1 and TH2 subsets of CD4+ T cells in peripheral blood of colorectal cancer patients and involvement in cancer establishment and progression.** *Cancer Immunol Immunother* 1996, **42**:1-8.
 37. Yoong KF, Adams DH: **Interleukin 2 restores CD3- ζ chain expression but fails to generate tumour-specific lytic activity in tumour-infiltrating lymphocytes derived from human colorectal hepatic metastases.** *Br J Cancer* 1998, **77**:1072-1081.
 38. Luo JS, Kammerer R, Schultze H, von Kleist S: **Modulations of the effector function and cytokine production of human lymphocytes by secreted factors derived from colorectal-carcinoma cells.** *Int J Cancer* 1997, **72**:142-148.
 39. Lindauer M, Rudy W, Guckel B, Doeberitz MV, Meuer SC, Moebius U: **Gene transfer of costimulatory molecules into a human colorectal cancer cell line: requirement of CD54, CD80 and class II MHC expression for enhanced immunogenicity.** *Immunology* 1998, **93**:390-397.
 40. Harashima N, Tanaka K, Sasatomi T, Shimizu K, Miyagi Y, Yamada A, Tamura M, Yamana H, Itoh K, Shichijo S: **Recognition of the Lck tyrosine kinase as a tumor antigen by cytotoxic T lymphocytes of cancer patients with distant metastases.** *Eur J Immunol* 2001, **31**:323-32.
 41. Mortarini R, Piris A, Maurichi A, Molla A, Bersani I, Bono A, Bartoli C, Santinami M, Lombardo C, Ravagnani F, Cascinelli N, Parmiani G, Anichini A: **Lack of terminally differentiated tumor-specific CD8+ T cells at tumor site in spite of antitumor immunity to self-antigens in human metastatic melanoma.** *Cancer Res* 2003, **63**:2535-45.
 42. Parmiani G, Sensi M, Castelli C, Rivoltini L, Anichini A: **T-cell response to unique and shared antigens and vaccination of cancer patients.** *Cancer Immunol* 2002, **2**:6.
 43. Marincola FM, Jaffee EM, Hicklin DJ, Ferrone S: **Escape of human solid tumors from T cell recognition: molecular mechanisms and functional significance.** *Adv Immunol* 2000, **74**:181-273.

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