Subproject 13: Dendritic cells in cancer immunotherapy

Principal Investigator: *José Alexandre Marzagão Barbuto*

Abstract

Cancer immunotherapy, investigated for a long time, but considered, till recently, unable to reach the expected success, acquired the status of one of the most relevant advances in current biomedical research (Couzin-Frankel, 2013; McNutt, 2013; Gravitz, 2013). This came to be due to the more precise understanding of the immune mechanisms involved in the development of cancer, and to the availability of new tools for their modulation. Nevertheless, though the pathways exploited today are reaching relative success, it is still possible to aim at a broader commitment of the immune response specificity and memory, inherent characteristics of such responses (Lanzavecchia and Sallusto, 2000). These are more efficiently exploited in immunization strategies against cancer – the therapeutic vaccines, which, however, did not reach yet their full ideal potential.

Effective anti-tumor vaccines have been very difficult to obtain (Barbuto, 2013), since, during the development of cancer, the neoplastic tissue and the body (specifically the immune system in this context) reach an equilibrium that allows the survival of neoplastic cells in an individual, theoretically at least, able to recognize and eliminate them (Dunn et al., 2004). This equilibrium depends on various tumor escape mechanisms (Lissoni et al., 1999; Sharma et al., 1999; Ohm and Carbone, 2001; Thurnher et al., 2001; Kusmartsev and Gabrilovich, 2002; Hoffmann et al., 2002; Yang et al., 2003; Baleeiro et al., 2008a), among which, the disturbance of antigenic presentation (Baleeiro et al., 2008b; Baleeiro and Barbuto, 2008; Ramos et al., 2012) is a quite effective strategy: without antigen presentation there is no immune response! Therefore, anti-tumor vaccines that attempt to induce an immune response in patients, in whom the presentation of tumor antigens has been biased by the development of the cancer, met barriers that, till recently, were practically impossible to cross.

Yet, the description of the dendritic cells (DCs) (Steinman and Witmer, 1978) and the realization that antigen presentation must be done by these cells (Steinman and Witmer, 1978; Steinman et al., 1983; Banchereau and Steinman, 1998; Steinman et al., 1999; Steinman, 2012) in order to a primary immune response to occur, opened new possibilities for the development of active immunization strategies against neoplasia. DCs are able to break already established immunological tolerance (Boog et al., 1985) and, since it became possible to differentiate these cells from blood precursors (Sallusto and Lanzavecchia, 1994), one can design therapeutic strategies that exploit them, in various clinical settings, including cancer (Barbuto et al., 2004), where, sometimes, encouraging results have been achieved (Dall'Oglio et al., 2003).

However, DCs constitute an extremely heterogeneous population and they are very sensitive to environment changes in the body. In cancer patients, DCs within the tumor

(Baleeiro et al., 2008a) and those derived from blood monocytes (Ramos et al., 2012; 2013) present many functional biases, which could explain, often, the disease progression or response to therapy. Thus, this study intends to evaluate, both in experimental models and in cancer patients, DCs within tumors and those derived from blood monocytes.

Specific Goals

Specifically, the project intends to:

1. Evaluate DCs in the tumor infiltrate under the various treatment/conditions as to:

a. Their membrane phenotype;

b. Their cytokine secretion pattern, spontaneous or induced by different stimuli, including tumor extracts;

c. Their ability to stimulate T lymphocytes, considering their response patterns (Th1, Th2, Th17, Treg);

2. Evaluate tumor infiltrating macrophages in the same conditions (considering the close proximity of these cell types) as to the same parameters above described, aiming mainly, at the characterization of the macrophages as to the M1 and M2 patterns;

3. Evaluate, before and after their inclusion in the different treatments, in the patients' blood:

a. The frequency of the various leukocyte subpopulations (CD4+, CD8+, FoxP3+ T lymphocytes, NK cells, B lymphocytes, dendritic cells, monocytes and granulocytes);

b. The monocytes' in vitro differentiation (evaluating, phenotypically and functionally the dendritic cells from them derived);

4. Study, in vitro, the effects of the various treatments over monocytes and monocyte-derived dendritic cells, evaluating:

a. Global gene expression of mRNAs and microRNAs (microarrays or NGS);

b. Protein expression profile (by proteomic techniques);

c. Cell function (preferential stimulation of lymphocyte subpopulations, cytokine production, immunoregulatory/immunostimulatory potential).

Goals

Dendritic cells act in the maintenance of homeostasis mainly through their ability to translate local disturbances into effective stimuli for the activation of lymphocytes. The suitability of the lymphocyte responses to the disequilibrium detected and presented by the dendritic cells within tissues is a decisive factor, between disease and return to the

homeostatic equilibrium. In cancer, a biased equilibrium is established, which allow tumors to grow unchecked by the immune system. Much of this bias can be traced back to functional deviations of dendritic cells in the patients. Thus, the present project intends to map, as broadly as possible the status of dendritic cells within tumor and of monocyte-derived dendritic cells, as well as the modifications of these cells induced by the various treatments/interventions planned within the global project. This mapping should lead to a better understanding of immune system-tumor interactions and thus, might help to improve immunotherapeutic approaches against cancer. A secondary and more specific aim of the project is the identification, if possible, of molecular targets for specifically designed therapeutic strategies that would "rescue" the DCs in cancer patients from their functional biases, thus allowing the immune system to act more effectively against cancer in the patients.

Implementation schedule

	1^{o} Year				**2^{o} Year**				**3^{o} Year**			
Activity / Months	1-5	6	7-11	12	13-17	18	19-23	24	25-29	30	31-35	36
Sample collection: tumor and/or blood, before/after interventions												
DC characterization within tumors: separation, phenotyping, functional evaluation												
Macrophage characterization within tumors: separation, phenotyping, functional evaluation												
Blood leukocytes subpopulation evaluation												
Study of monocyte-derived dendritic cells in cancer patients: phenotype and function												
Functional evaluation of monocytes and monocyte-derived dendritic cells before and after different interventions												
mRNA and microRNA evaluation in monocytes and monocyte-derived dendritic cells before and after different interventions												
Protein expression profile in monocytes and monocyte-derived dendritic cells before and after different interventions												

Interim data analysis												
Final data analysis												
Scientific manuscripts preparation												

	4º Year				5º Year				6º Year			
Activity / Months	**37-41**	**42**	**43-47**	**48**	**49-53**	**54**	**55-59**	**60**	**61-65**	**66**	**67-71**	**72**
Sample collection: tumor and/or blood, before/after interventions												
DC characterization within tumors: separation, phenotyping, functional evaluation												
Macrophage characterization within tumors: separation, phenotyping, functional evaluation												
Blood leukocytes subpopulation evaluation												
Study of monocyte-derived dendritic cells in cancer patients: phenotype and function												
Functional evaluation of monocytes and monocyte-derived dendritic cells before and after different interventions												
mRNA and microRNA evaluation in monocytes and monocyte-derived dendritic cells before and after different interventions												
Protein expression profile in monocytes and monocyte-derived dendritic cells before and after different interventions												
Interim data analysis												
Final data analysis												
Scientific manuscripts preparation												

Researchers:

Adilson Kleber Ferreira

Ana Carolina Franco Ferreira

Patrícia Cruz Bergami-Santos

Ricardo Alexandre Azevedo

Salomão Dória Jorge

References

Baleeiro, R.B., and J.A.M. Barbuto. 2008. Local secretion/shedding of tumor-derived CD83 molecules as a novel tumor escape mechanism. *Mol. Immunol.* 45:3502–3504. doi:10.1016/j.molimm.2008.04.005.

Baleeiro, R.B., L.B. Anselmo, F.A. Soares, C.A.L. Pinto, O. Ramos, J.L. Gross, F. Haddad, R.N. Younes, M.Y. Tomiyoshi, P.C. Bergami-Santos, and J.A.M. Barbuto. 2008a. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. *Cancer Immunol. Immunother.* 57:1335–1345. doi:10.1007/s00262-008-0468-7.

Baleeiro, R.B., P.C. Bergami-Santos, M.Y. Tomiyoshi, J.L. Gross, F. Haddad, C.A.L. Pinto, F.A. Soares, R.N. Younes, and J.A.M. Barbuto. 2008b. Expression of a dendritic cell maturation marker CD83 on tumor cells from lung cancer patients and several human tumor cell lines: is there a biological meaning behind it? *Cancer Immunol. Immunother.* 57:265–270. doi:10.1007/s00262-007-0344-x.

Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature*. 392:245–252. doi:10.1038/32588.

Barbuto, J.A.M. 2013. Are dysfunctional monocyte-derived dendritic cells in cancer an explanation for cancer vaccine failures? *Immunotherapy*. 5:105–107. doi:10.2217/imt.12.153.

Barbuto, J.A.M., L.F.C. Ensina, A.R. Neves, P.C. Bergami-Santos, K.R.M. Leite, R. Marques, F. Costa, S.C. Martins, L.H. Câmara-Lopes, and A.C. Buzaid. 2004. Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. *Cancer Immunol. Immunother.* 53:1111–1118. doi:10.1007/s00262-004-0551-7.

Boog, C.J., W.M. Kast, H.T. Timmers, J. Boes, L.P. de Waal, and C.J. Melief. 1985. Abolition of specific immune response defect by immunization with dendritic cells. *Nature*. 318:59–62.

Couzin-Frankel, J. 2013. Cancer Immunotherapy. *Science*. 342:1432–1433. doi:10.1126/science.342.6165.1432.

Dall'Oglio, M.F., M. Srougi, and J.A.M. Barbuto. 2003. Complete response of metastatic renal cancer with dendritic cell vaccine. *Int Braz J Urol*. 29:517–519.

Dunn, G.P., L.J. Old, and R.D. Schreiber. 2004. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 21:137–148.

doi:10.1016/j.immuni.2004.07.017.

Gravitz, L. 2013. Cancer immunotherapy. *Nature*. 504:S1–S1. doi:10.1038/504S1a.

Hoffmann, T.K., J. Muller-Berghaus, R.L. Ferris, J.T. Johnson, W.J. Storkus, and T.L. Whiteside. 2002. Alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinomas of the head and neck. *Clin. Cancer Res.* 8:1787–1793.

Kusmartsev, S., and D.I. Gabrilovich. 2002. Immature myeloid cells and cancer-associated immune suppression. *Cancer Immunol. Immunother.* 51:293–298. doi:10.1007/s00262-002-0280-8.

Lanzavecchia, A., and F. Sallusto. 2000. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science*. 290:92–97.

Lissoni, P., L. Vigore, R. Ferranti, R. Bukovec, S. Meregalli, M. Mandala, S. Barni, G. Tancini, L. Fumagalli, and L. Giani. 1999. Circulating dendritic cells in early and advanced cancer patients: diminished percent in the metastatic disease. *J Biol Regul Homeost Agents*. 13:216–219.

McNutt, M. 2013. Cancer immunotherapy. *Science*. 342:1417–1417. doi:10.1126/science.1249481.

Ohm, J.E., and D.P. Carbone. 2001. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res*. 23:263–272. doi:10.1385/IR:23:2-3:263.

Ramos, R.N., C.J. de Moraes, B. Zelante, and J.A.M. Barbuto. 2013. What are the molecules involved in regulatory T-cells induction by dendritic cells in cancer? *Clin. Dev. Immunol.* 2013:806025–10. doi:10.1155/2013/806025.

Ramos, R.N., L.S. Chin, A.P.S.A. Azevedo, P.C. Bergami-Santos, F. Laginha, and J.A.M. Barbuto. 2012. Monocyte-derived dendritic cells from breast cancer patients are biased to induce CD4+CD25+Foxp3+ regulatory T cells. *J. Leukoc. Biol.* 92:673–682. doi:10.1189/jlb.0112048.

Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* 179:1109–1118.

Sharma, S., M. Stolina, Y. Lin, B. Gardner, P.W. Miller, M. Kronenberg, and S.M. Dubinett. 1999. T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *The Journal of Immunology*. 163:5020–5028.

Steinman, R.M. 2012. Decisions about dendritic cells: past, present, and future. *Annu. Rev. Immunol.* 30:1–22. doi:10.1146/annurev-immunol-100311-102839.

Steinman, R.M., and M.D. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natl. Acad. Sci. U.S.A.* 75:5132–5136.

Steinman, R.M., B. Gutchinov, M.D. Witmer, and M.C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613–627.

Steinman, R.M., K. Inaba, S. Turley, P. Pierre, and I. Mellman. 1999. Antigen capture,

processing, and presentation by dendritic cells: recent cell biological studies. *Hum Immunol*. 60:562–567.

Thurnher, M., C. Zelle-Rieser, R. Ramoner, G. Bartsch, and L. Holtl. 2001. The disabled dendritic cell. *FASEB J*. 15:1054–1061.

Yang, L., N. Yamagata, R. Yadav, S. Brandon, R.L. Courtney, J.D. Morrow, Y. Shyr, M. Boothby, S. Joyce, D.P. Carbone, and R.M. Breyer. 2003. Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP2 receptor. *Journal of Clinical Investigation*. 111:727–735. doi:10.1172/JCI16492.

Subprojeto 13: Células dendríticas na imunoterapia do câncer

Pesquisador Responsável: *José Alexandre Marzagão Barbuto*

Introdução

A imunoterapia do câncer, por muito tempo investigada mas considerada, até recentemente, incapaz de atingir o sucesso esperado, passou a ser vista como um dos avanços mais relevantes da pesquisa biomédica atual (Couzin-Frankel, 2013; McNutt, 2013; Gravitz, 2013). Isto veio se tornando possível graças à compreensão mais precisa dos mecanismos imunes envolvidos no desenvolvimento das neoplasias, bem como pela disponibilização de novos instrumentos de modulação destes. Todavia, embora os mecanismos hoje explorados venham alcançando sucesso relativo, ainda se pode almejar a explorar mais amplamente a especificidade e a memória da resposta imune, características inerentes da mesma (Lanzavecchia and Sallusto, 2000). Estas características são exploradas mais eficazmente em estratégias de imunização contra o câncer – as vacinas terapêuticas – que, no entanto, ainda não atingiram todo seu potencial ideal.

Vacinas anti-tumorais eficazes têm sido muito difíceis de conseguir (Barbuto, 2013), uma vez que, durante o desenvolvimento do câncer, o tecido neoplásico e organismo (especificamente o sistema imune neste contexto) atingem um equilíbrio que permite a sobrevivência das células neoplásicas num indivíduo, teoricamente ao menos, capaz de reconhecer e eliminar as mesmas (Dunn et al., 2004). Este equilíbrio depende de diversos mecanismos de escape do tumor (Lissoni et al., 1999; Sharma et al., 1999; Ohm and Carbone, 2001; Thurnher et al., 2001; Kusmartsev and Gabrilovich, 2002; Hoffmann et al., 2002; Yang et al., 2003; Baleeiro et al., 2008a), dentre os quais a perturbação da apresentação antigênica (Baleeiro et al., 2008b; Baleeiro and Barbuto, 2008; Ramos et al., 2012) representa uma estratégia bastante eficaz: sem apresentação antigênica não há resposta imune! Portanto, vacinas anti-tumorais, que pretendam induzir uma resposta imune em pacientes onde a apresentação de antígenos tumorais foi "desvirtuada" pelo desenvolvimento do câncer, encontram barreiras até há pouco, praticamente impossíveis de transpor.

Contudo, com a descrição das células dendríticas (DCs) (Steinman and Witmer, 1978) e o reconhecimento de que a apresentação antigênica deve ser feita por tais células (Steinman and Witmer, 1978; Steinman et al., 1983; Banchereau and Steinman, 1998; Steinman et al., 1999; Steinman, 2012) para que a resposta imune primária ocorra, abriram novas possibilidades para o desenvolvimento de estratégias de imunização ativa contra as neoplasias. DCs são capazes de quebrar estados de tolerância imunológica já estabelecidos (Boog et al., 1985) e, desde que se tornou possível diferenciar este tipo celular a partir de precursores sangüíneos (Sallusto and Lanzavecchia, 1994), pode-se desenhar estratégias terapêuticas que as explorem, em diversas situações clínicas, inclusive no câncer (Barbuto et al., 2004), onde, por vezes, se conseguem resultados animadores (Dall'Oglio et al., 2003).

Porém, as DCs são uma população extremamente heterogênea e muito sensíveis às variações ambientais no indivíduo. Em pacientes com câncer, tanto as presentes no tumor (Baleeiro et al., 2008a), quanto as derivadas de monócitos circulantes (Ramos et al., 2012; 2013), apresentam diversas alterações funcionais que podem explicar, muitas vezes, a evolução aparentemente da doença ou da resposta clínica dos pacientes a diferente tratamentos. Assim, a proposta deste estudo é avaliar, tanto em modelos experimentais quanto em pacientes portadores de neoplasias, DCs, integrantes dos tecidos neoplásicos ou derivadas de monócitos.

Objetivo Geral

Especificamente pretende-se:

1. *Avaliar as DCs do infiltrado celular em tumores submetidos aos diferentes tratamentos:*
    a. *Quanto a seu fenótipo de membrana;*
    b. *Quanto a seu padrão de produção de citocinas espontâneo e induzido por diversos estimulantes, inclusive extratos tumorais;*
    c. *Quanto à sua capacidade de estimulação linfocitária, considerando os padrões de resposta dos mesmos (Th1, Th2, Th17, Treg);*
2. *Avaliar os macrófagos presentes no infiltrado celular dos mesmos tumores (considerando a proximidade ontogênica e funcional destes tipos celulares) quanto aos mesmos parâmetros acima descritos, buscando-se, principalmente, a caracterização dos mesmos quanto aos padrões M1 e M2;*
3. *Avaliar, antes e após sua inclusão nos diferentes tratamentos, no sangue dos pacientes:*
    a. *A frequência de subpopulações de leucócitos (linfócitos T CD4+, CD8+, FoxP3+, NK, B, células dendríticas, monócitos e granulócitos);*
    b. *A capacidade de diferenciação in vitro de monócitos (avaliando fenotípica- e funcionalmente células dendríticas deles derivadas;*
4. *Estudar, in vitro, os efeitos dos diferentes tratamentos sobre monócitos e sobre células dendríticas derivadas de monócitos, avaliando-se:*
    a. *A expressão gênica global de mRNAs e microRNAs (microarrays ou NGS);*
    b. *O perfil de expressão proteica (por técnicas de protêomica);*
    c. *A função celular (estimulação preferencial de subpopulações de linfócitos, produção de citocinas, potencial imunorregulador/imunoestimulador).*

Metas

As células dendríticas (DCs) atuam na manutenção da homeostase do organismo, e que por muitas vezes, o fazem através da ativação de linfócitos. A expansão clonal dos linfócitos ocorre em resposta, a um padrão de resposta de acordo com os estímulos que recebe do ambiente. Neste senário, a investigação de como as DCs, podem prover efeitos supressores dos linfócitos no contexto das neoplasias, e a investigação de como o tumor estabelece um estado de tolerância ao tumor, será a meta central deste subprojeto. Ademais, o foco se estenderá, além da meta central, pretende-se também elucidar como o tumor pode influenciar no fenótipo da DCs, e quais as moléculas envolvidas nesta modulação. Ao final espera-se identificar tais moléculas, e se estas, podendo-se, ser considerados como novos alvos moleculares com objetivo de desenvolver abordagens terapêuticas mais eficazes para imunoterapia do câncer.

Cronograma de execução referente a seis anos de projeto

	1º Ano				**2º Ano**				**3º Ano**			
Atividade / Meses	1-5	6	7-11	12	13-17	18	19-23	24	25-29	30	31-35	36
Coleta de material de pacientes: tumor e/ou sangue, antes e pós tratamentos												
Estudo da DC do infiltrado tumoral: Separação, Fenotipagem, avaliação funcional												
Estudo da Macrófago do infiltrado tumoral: Separação, Fenotipagem, avaliação funcional												
Avaliação das subpopulações de Leucócitos no sangue dos pacientes												
Estudo das DCS derivadas de monócitos isolados do sangue de pacientes: Fenótipo e função												
Avaliação funcional de monócitos e DCS derivadas de monócitos antes e após os diferentes tratamentos												
Avaliação da expressão de mRNAs e microRNAs em monócitos e DCS derivadas de monócitos antes e após os tratamentos												
Avaliação do perfil de expressão proteica em monócitos e DCS derivadas de monócitos antes e após os tratamentos												
Compilação e análise de resultados parciais												
Compilação e análise dos resultados finais e elaboração do relatório final												
Elaboração de manuscrito científico												

	4º Ano	**5º Ano**	**6º Ano**

Atividade / Meses	37-41	42	43-47	48	49-53	54	55-59	60	61-65	66	67-71	72
Coleta de material de pacientes: tumor e/ou sangue, antes e pós tratamentos												
Estudo da DC do infiltrado tumoral: Separação, Fenotipagem, avaliação funcional												
Estudo da Macrófago do infiltrado tumoral: Separação, Fenotipagem, avaliação funcional												
Avaliação das subpopulações de Leucócitos no sangue dos pacientes												
Estudo das DCS derivadas de monócitos isolados do sangue de pacientes: Fenótipo e função												
Avaliação funcional de monócitos e DCS derivadas de monócitos antes e após os diferentes tratamentos												
Avaliação da expressão de mRNAs e microRNAs em monócitos e DCS derivadas de monócitos antes e após os tratamentos												
Avaliação do perfil de expressão proteica em monócitos e DCS derivadas de monócitos antes e após os tratamentos												
Compilação e ánalise de resultados parciais												
Compilação e ánalise dos resultados finais e elaboração do relatorio final												
Elaboração de manuscrito científico												

Pesquisadores:

Adilson Kleber Ferreira
Ana Carolina Franco Ferreira
Patrícia Cruz Bergami-Santos
Ricardo Alexandre Azevedo
Salomão Dória Jorge

Referências

Baleeiro, R.B., and J.A.M. Barbuto. 2008. Local secretion/shedding of tumor-derived CD83 molecules as a novel tumor escape mechanism. *Mol. Immunol.* 45:3502–3504. doi:10.1016/j.molimm.2008.04.005.

Baleeiro, R.B., L.B. Anselmo, F.A. Soares, C.A.L. Pinto, O. Ramos, J.L. Gross, F. Haddad, R.N. Younes, M.Y. Tomiyoshi, P.C. Bergami-Santos, and J.A.M. Barbuto. 2008a. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. *Cancer Immunol. Immunother.* 57:1335–1345.

doi:10.1007/s00262-008-0468-7.

Baleeiro, R.B., P.C. Bergami-Santos, M.Y. Tomiyoshi, J.L. Gross, F. Haddad, C.A.L. Pinto, F.A. Soares, R.N. Younes, and J.A.M. Barbuto. 2008b. Expression of a dendritic cell maturation marker CD83 on tumor cells from lung cancer patients and several human tumor cell lines: is there a biological meaning behind it? *Cancer Immunol. Immunother.* 57:265–270. doi:10.1007/s00262-007-0344-x.

Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature*. 392:245–252. doi:10.1038/32588.

Barbuto, J.A.M. 2013. Are dysfunctional monocyte-derived dendritic cells in cancer an explanation for cancer vaccine failures? *Immunotherapy*. 5:105–107. doi:10.2217/imt.12.153.

Barbuto, J.A.M., L.F.C. Ensina, A.R. Neves, P.C. Bergami-Santos, K.R.M. Leite, R. Marques, F. Costa, S.C. Martins, L.H. Câmara-Lopes, and A.C. Buzaid. 2004. Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. *Cancer Immunol. Immunother.* 53:1111–1118. doi:10.1007/s00262-004-0551-7.

Boog, C.J., W.M. Kast, H.T. Timmers, J. Boes, L.P. de Waal, and C.J. Melief. 1985. Abolition of specific immune response defect by immunization with dendritic cells. *Nature*. 318:59–62.

Couzin-Frankel, J. 2013. Cancer Immunotherapy. *Science*. 342:1432–1433. doi:10.1126/science.342.6165.1432.

Dall'Oglio, M.F., M. Srougi, and J.A.M. Barbuto. 2003. Complete response of metastatic renal cancer with dendritic cell vaccine. *Int Braz J Urol*. 29:517–519.

Dunn, G.P., L.J. Old, and R.D. Schreiber. 2004. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 21:137–148. doi:10.1016/j.immuni.2004.07.017.

Gravitz, L. 2013. Cancer immunotherapy. *Nature*. 504:S1–S1. doi:10.1038/504S1a.

Hoffmann, T.K., J. Muller-Berghaus, R.L. Ferris, J.T. Johnson, W.J. Storkus, and T.L. Whiteside. 2002. Alterations in the frequency of dendritic cell subsets in the peripheral circulation of patients with squamous cell carcinomas of the head and neck. *Clin. Cancer Res.* 8:1787–1793.

Kusmartsev, S., and D.I. Gabrilovich. 2002. Immature myeloid cells and cancer-associated immune suppression. *Cancer Immunol. Immunother.* 51:293–298. doi:10.1007/s00262-002-0280-8.

Lanzavecchia, A., and F. Sallusto. 2000. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. *Science*. 290:92–97.

Lissoni, P., L. Vigore, R. Ferranti, R. Bukovec, S. Meregalli, M. Mandala, S. Barni, G. Tancini, L. Fumagalli, and L. Giani. 1999. Circulating dendritic cells in early and advanced cancer patients: diminished percent in the metastatic disease. *J Biol Regul Homeost Agents*. 13:216–219.

McNutt, M. 2013. Cancer immunotherapy. *Science*. 342:1417–1417. doi:10.1126/science.1249481.

Ohm, J.E., and D.P. Carbone. 2001. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res*. 23:263–272. doi:10.1385/IR:23:2-3:263.

Ramos, R.N., C.J. de Moraes, B. Zelante, and J.A.M. Barbuto. 2013. What are the molecules involved in regulatory T-cells induction by dendritic cells in cancer? *Clin. Dev. Immunol.* 2013:806025–10. doi:10.1155/2013/806025.

Ramos, R.N., L.S. Chin, A.P.S.A. Azevedo, P.C. Bergami-Santos, F. Laginha, and J.A.M. Barbuto. 2012. Monocyte-derived dendritic cells from breast cancer patients are biased to induce CD4+CD25+Foxp3+ regulatory T cells. *J. Leukoc. Biol.* 92:673–682. doi:10.1189/jlb.0112048.

Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J. Exp. Med.* 179:1109–1118.

Sharma, S., M. Stolina, Y. Lin, B. Gardner, P.W. Miller, M. Kronenberg, and S.M. Dubinett. 1999. T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *The Journal of Immunology*. 163:5020–5028.

Steinman, R.M. 2012. Decisions about dendritic cells: past, present, and future. *Annu. Rev. Immunol.* 30:1–22. doi:10.1146/annurev-immunol-100311-102839.

Steinman, R.M., and M.D. Witmer. 1978. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natl. Acad. Sci. U.S.A.* 75:5132–5136.

Steinman, R.M., B. Gutchinov, M.D. Witmer, and M.C. Nussenzweig. 1983. Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 157:613–627.

Steinman, R.M., K. Inaba, S. Turley, P. Pierre, and I. Mellman. 1999. Antigen capture, processing, and presentation by dendritic cells: recent cell biological studies. *Hum Immunol*. 60:562–567.

Thurnher, M., C. Zelle-Rieser, R. Ramoner, G. Bartsch, and L. Holtl. 2001. The disabled dendritic cell. *FASEB J*. 15:1054–1061.

Yang, L., N. Yamagata, R. Yadav, S. Brandon, R.L. Courtney, J.D. Morrow, Y. Shyr, M. Boothby, S. Joyce, D.P. Carbone, and R.M. Breyer. 2003. Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP2 receptor. *Journal of Clinical Investigation*. 111:727–735. doi:10.1172/JCI16492.