Immunotherapies Towards Tumor Initiating Cells and Cancer Stem Cells

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Abstract: Active immunotherapy of cancer is a promising treatment modality by inducing long lived tumor reactive immune effector cells. Several vaccine trials have indicated that its effectiveness is probably best when the tumor burden is low. An attractive population of cells to target by this approach may be tumor initiating cells (TICs). Recent research suggests that such types of cells may be precursors to cancer possibly giving rise to metastatic disease. Although more work is being done to characterize appropriate phenotypic and functional markers, this population of cells would make an ideal target for active immunotherapy in high risk patients, so as to achieve remission over much longer periods of time. This review gives an overview of the potential of immunotherapy and how it may target TICs as a potential treatment option for cancer patients.

INTRODUCTION

Tumor immunology has developed into a distinct entity over the past 2 decades with several key laboratory vaccine concepts being tested as therapies in cancer patients. While several types of cancer vaccines have demonstrated successful eradication of tumors in preclinical models, this promise has not translated as effectively into the clinic. The immune system is believed to have evolved to recognize 'nonself' antigens present on invading pathogens. Cancers, on the other hand, arise from 'self' tissue and are not efficient in inducing a strong enough immune response to keep the tumor in check or eradicate them. However, it is well known that tumor immunity exists, perhaps from recognizing epitopes from 'altered' self proteins [1]. Examples of spontaneous regressions have been documented in some solid tumors [2] with infiltrating lymphocytes in the tumor bed of ovarian carcinomas correlating with improved prognosis [3]. These examples indicate the presence of immune reactive cells that can potentially reject tumors.

With the results from several pivotal cancer vaccine trials, it has become increasingly evident that a low tumor burden is an important point for vaccines to be effective. Tumor mediated immune suppressive mechanisms such as those seen due to TGF-β and T-reg cells would be low, providing an environment that could be conducive for optimal manipulation of the immune system. Another important aspect is the choice of relevant targets. These may be phenotypic or functional markers on cancer cells, precursors or tumor initiating cells. Among the choice of appropriate targets to treat or prevent a recurrence, an area that is increasingly gaining importance is tumor initiating cells (TICs). Several types of TICs have been described in various studies such as cancer stem cell (CSC) [4], side population and precursor cells, and mature cells [5, 6], which have undergone transformation.

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CSCs have been hypothesized in several cancers including leukemias, breast, brain, colon, prostate, pancreas, and are thought to be important players in the incidence of metastases and recurrence of tumors *in situ*. They are thought to be a quiescent population of cells of up to about 0.01% of a tumor that possibly exhibit chemo- and radio-resistance [7]. In addition to this population, other types of cells such as dominant clones within the tumor may also participate in tumor progression and recurrence [8], and it is probable that most tumors may have combinations of both these cell types which participate in the manifestation of the cancer. Thus any therapy targeting these cells that can initiate a cancer may be an adjunct therapy that will be administered in addition to conventional and novel therapeutics targeting other facets of the tumor. It may also be used as a vaccine in the adjuvant setting to confer long term protection from a recurrence.

For the sake of simplicity in discussing the nature and potential of a population of cells that can give rise to cancer and serve as a potential target of immunotherapy, we will adhere to the term tumor initiating cells (TICs) from now on.

Targeted Immunotherapy of Cancers

The vast majority of chemotherapeutics is indiscriminate in their cytotoxic action on a dividing cell, affecting healthy dividing cells as well as cancer cells. However, as a result of an increasing understanding of how cancer cells work, a handful of new molecules on cancer cells were identified and targeted, so as to minimize unwanted side effects. In addition to existing targeted chemotherapies, a few biologics have also been developed. In passive immunotherapy, antibodies targeting specific cell surface markers on cancer cells or receptor ligands are administered (described below). The effect of the therapeutic is short lived, making repeated treatments necessary. In contrast, active immunotherapy or vaccines engage the immune system to specifically target antigen expressing tumors. They can create a memory pool of immune effectors that will potentially allow for long lived surveillance of new tumors bearing the same targets. Below is a short description showing the potential of both passive and

1876-4010/08

active immunotherapies of cancer in treatment and prevention settings.

Passive Immunotherapy

Targeted immunotherapies have been successfully developed in the antibody realm. So far, they have been restricted to the development of antibodies to various cancer and stromal associated molecules. Over the past decade, several monoclonal antibodies have been approved as passive immunotherapeutics. These antibodies are being used in treatment regimens either singly or in combination with other targeted chemotherapies. Largely, they are those that bind to members of the growth factor receptor family. Examples are Erbitux® (Imclone Systems, Inc) and Herceptin (Genentech, Inc) where they bind the extracellular domain of the receptor and prevent its ligand from engaging the receptor to induce downstream signaling and proliferation. Erbitux® is used to treat advanced colon cancer and acts by targeting the EGF receptor (EGFR) either as a single agent or in combination with irinotecan. Herceptin® is the other targeted antibody used in the treatment of HER2-positive breast cancer in women with stage II, III or IV also as a single agent or in combination with paclitaxel. Both EGFR and HER2 are found to be overexpressed in colon carcinomas and breast cancer, respectively, and therefore make suitable targets. Avastin®, on the other hand, targets circulating VEGF ligand thereby reducing the availability of the ligand and the probability of engaging VEGFR to initiate angiogenesis. In combination with 5-fluorouracil chemotherapy Avastin® is used in the treatment of metastatic colon and rectal carcinoma. Radiolabelled antibodies have also been successful in targeting B-cell lymphomas. Noteworthy ones are Bexxar™ (Corixa, Inc/Glaxo-Smith Kline, Inc) and Zevalin ® (Biogen-Idec, Inc) which are radiolabelled monoclonal antibodies to CD20, to treat relapsed or refractory low grade, follicular or transformed B-cell non-Hodgkin's lymphoma, while Rituxan™ (Biogen-Idec, Inc) is an unlabelled monoclonal used to target CD20 among positive, refractory non-Hodgkin's Lymphoma (NHL) patients. In the case of radiolabeled antibodies, the attack on the tumor cell is probably two-pronged. The antibody carries the radioisotope to the cancer where it is destroyed by radiation. In addition, some of these antibodies may also exhibit a cytotoxic activity on the cancer cell.

Active Immunotherapy

Active immunotherapies or cancer vaccines have not seen the same level of success as antibodies. However, there is still widespread belief for the potential and utility of harnessing the patient's immune system to recognize and keep the cancer from recurring. With over half a dozen pivotal trials that have not been successful in the recent past, several criteria pertaining to all spheres of research and development have come to light providing guidelines that may help in the progress of generating a successful cancer vaccine [9-14]. A list showing a variety of vaccines that were clinically tried (and some ongoing) are shown in Table **1**. This is not a complete list of all cancer vaccines that have been tried or are ongoing, but one that shows a variety of vaccines that have reached a pivotal trial [15-36]. Of all the vaccines to date, the furthest along that may be close to licensure is Provenge®, an autologous vaccine pulsed with PAP for treatment of asymptomatic, metastatic, androgen-independent prostate cancer. At the time of filing a BLA for Provenge®, the study showed that vaccinated patients showed a survival time of about 4.5 months longer than the placebo treated control group with a 41% overall reduction in the risk of death. However, the FDA requested additional clinical data in support of the BLA filing. The planned interim results of this trial just received indicated a 20% reduction in the risk of death in the vaccine arm relative to the placebo arm (http://investor.dendreon.com/ReleaseDetail.cfm?ReleaseID=338495&Header=News). There were no safety concerns and the trial is expected to continue to its final analysis.

Several factors have emerged from these trials that may be considered for further development of vaccines and these may be scientific, clinical, or logistical in nature. In the preclinical area, identification of relevant target antigens (both on the tumor and stroma) that are specific and relevant to biology of the tumor should be considered. A variety of antigens have been identified to which either spontaneous or induced immune responses have been reported in several studies [37]. Although immunization with these antigens have been known to protect mice from subsequent syngeneic tumor challenge, as well as induce a detectable T cell or antibody response in vaccinated patients, these data have not correlated with a desired clinical outcome. Lessons from some of the successful therapies have taught us that targeting those molecules crucial for signaling/ proliferation and cell survival, or those that are overexpressed may be one method of arresting tumor spread. Examples of these are tyrosine kinase and proteosome inhibitors (Gleevac, Iressa and Tarceva, and Velcade, respectively) as chemotherapy, and antibodies to members of the growth factor receptor family (EGFR and HER2) and angiogenesis inducing ligand (VEGF). Erbitux, a chimeric monoclonal antibody, binds to the extracellular domain of the receptor and blocks binding of the ligand EGF to block downstream signaling and consequently growth and proliferation. Herceptin, a humanized monoclonal antibody, inhibits tumor growth by arresting cell cycle at the G1 phase as well as by disrupting downstream signaling, also leading to cell cycle arrest. Other factors to consider may be inclusion of suitable adjuvants in the vaccine regimen, other T cell co-stimulants, T-reg inhibitors and methods to down-regulate tumor suppressor factors [38]. The idea is to achieve a magnitude of anti-tumor response as that seen in an infectious disease setting. The greatest chance of achieving this success is most likely when some of these immunological agents are given in combination. However, it has become increasingly clear that this is also more achievable only when the tumor burden is minimal or there is no evidence of disease. In this scenario, there is less tumor induced immune suppression that could dampen an effective anti-tumor response.

While the above suggestions are broad indications for a successful cancer vaccine, a few of these can be more readily applied to that of a TIC setting. These will be addressed in the section below.

Tumor Initiating Cells as Potential Target for Immunotherapy

Cancer stem cells are one of the TICs which could be targets for vaccines. CSCs should possess the properties of proliferation and differentiation at least into the fates of the

Table 1. Examples of Cancer Vaccine Trials that are Either Discontinued or Ongoing

Vaccine	Nature of Vaccine	Disease	Trial Status	References
Melacine ® (Corixa)	Allogeneic whole cell tumor lysate+DETOX	Melanoma - Stage III, Stage IV	Discontd.	[15, 16]
PANVAC-VF (Therion)	Inactivated fowl pox virus encoding for costimulatory molecules ICAM-1, B-7.1, LFA-3, and CEA and MUC-1	Pancreatic cancer	Discontd.	[17]
Bec2 (Imclone)	Anti-Id to GD3	SCLC	Discontd.	[18]
Canvaxin™ (Cancer-Vax)	Allogeneic whole cell vaccine	Melanoma - Stage IV; Stage III	Discontd.	[19, 20]
Oncophage® (Antigen-ics)	Hsp96 (kidney cancer cells)	Renal cell carcinoma - Stage IV	Discontd.	[21]
Theratope® (Biomira)	Stn-KLH (MUC1)	Metastatic (Stage IV) breast cancer	Discontd.	[22]
MyVax (Genitope)	Patient specific recombinant idiotype protein coupled with KLH and injected with GM-CSF	Follicular non-Hodgkin's lymphoma	Discontd.	[23]
GVAX (Cell Genesys) – VITAL 2	Allogeneic prostate tumor cells transfected with GM-CSF	Hormone resistant prostate cancer (GVAX immunotherapy in combination with Taxotere chemotherapy vs. Taxotere and prednisone)	Discontd.	[24, 25]
GVAX (Cell Genesys) – VITAL 1	Allogeneic prostate tumor cells transfected with GM-CSF	Hormone resistant prostate cancer (GVAX immunotherapy vs. Taxotere chemotherapy and prednisone)	Ongoing	[26]
Lucanix ™ (NovaRx)	Allogeneic tumor cells tranfected with antisense TGFβ	Non small cell lung cancer	Ongoing	[27, 28]
Proteinase 3 PR1 peptide (The Vaccine Company)	Peptide + GM-CSF	Acute Myeloid Leukemia (AML)	Ongoing	[29-31]
Sipuleucel-T (Dendreon)	Autologous $CD54^+$ cells/PAP	Metastatic, hormone refractory, asymptomatic prostate cancer	Ongoing; interim results in Oct 2008	[32-36]

Table 1: A list of various types of cancer vaccines in pivotal trials that has provided valuable indications for the successful development of future vaccines (Discontd.; Discontinued)

tissue in which the tumor occurs. In addition serial transplantation into mouse xenograft models, which should result in formation of tumors which are similar to the parental tumor, is considered a functional assay for CSC. By these definitions, there have been several papers in the recent past which report that CSCs have been isolated from tumors of blood (specifically leukemias), brain, breast, colon, pancreas and prostate [39-47]. Table **2** lists the surface markers which have been used for this identification. The markers which have been used for isolation of these CSCs are not exclusive for CSCs, but are also found on normal stem cells, or even in other tissues. Furthermore clear markers identifying other types of TICs are also in the process of being defined.

Given the dearth of identifying markers on TICs, there is increasing reliance on functional properties for isolation and definition. Two frequently used experimental protocols used are the sphere forming assay and the xenograft assay. The sphere forming assay is reminiscent of the neurosphere assay which is one of the methods used to proliferate neural stem cells. As it selects for a population of proliferating cells which can grow without a substratum; these cells could potentially include any of the proliferative cells of the tumor, in addition to the CSCs. The xenograft model has served to prove that tumors can be serially passaged within mice, in order to demonstrate that tumors of similar histology can be propagated with putative CSCs. However, the propagation of human cells in mice might include a selection step where only selected cells could survive and proliferate in an alien atmosphere of a xenogeneic species. In addition, cells within the fraction thought to exclude CSCs have also been shown to cause tumors in the case of brain and colon CSCs [48, 49].

Table 2. Cancer Stem Cells and Associated Markers

Cancer	Markers used for isolation of CSCs	Reference
Blood (leukemia)	$CD34^+$; $CD38^-$	[39]
Brain	$CD133^+$	[40]
Breast	$CD24^{-/low}$; $CD44^+$	[41]
Colon	$CD133^+$ OR $CD44^+$; Lin^-; ESA^+	[42-44]
Pancreas	$CD133^+$ OR $CD44^+$; $CD24^+$; ESA^+	[45, 46]
Prostate	$CD44^+$; $\alpha2\beta1^{high}$; $CD133^+$	[47]

Table 2: A list of cancer stem cells and the markers that have been used for their isolation.

These recent conflicting observations relating to CSCs and their properties, does leave some room for evolution in current methods of identification of CSCs.

Markers specific for TICs are being sought for purposes of identification, and they will also prove useful for targeting these cells. Several other methods may be used to identify such surface markers including differential expression, genomics and proteomics approaches. In another approach it was determined whether markers known to occur in certain cancers are present in CSCs isolated from those specific tissues [50]. MUC1, a well known tumor marker, was present in cells selected as the 'side population cells' from MCF7, a breast cancer cell line. The 'side population' includes cells which group as a population of smaller cells with increased efficiency of dye efflux and which are thought to co-isolate with the CSC population. In another study of multiple myeloma (MM) immunity to the SOX2 antigen was expressed in patients with a precursor condition to MM called monoclonal gammopathy of undetermined significance (MGUS). This immunity was lost in patients where the disease had progressed although SOX2 positive cells remained suggesting a T-cell malfunction [51]. However, this study does suggest that SOX2 is a possible marker for some precursors.

In spite of the limitations noted above, the concept of TICs is an extremely attractive one to factor into cancer therapeutics. For effective use in developing an immunotherapy targeting these cells, identification of cells which could give rise to tumor mass is perhaps more important rather than specifying whether they are CSCs or precursors. Immunotherapies may be used to advantage in scenarios where the targets are not in overwhelmingly large numbers, and as such the idea of targeting TICs with immunotherapy is attractive because these cells are thought to be present at a very small fraction of a tumor. Since a precise set of markers which define TICs remains elusive, the targeting of these cancer forming cells for immunotherapies by the use of surface markers is still an evolving field. One possibility by which these cells may be targeted is by manipulation of the niche that they are present in, such that they are forced to exit from their quiescent state [7]. This could be especially relevant in the case of CSCs. A recent report indicates that HES1 causes cells to remain in quiescent state without inducing senescence [52]. Perhaps one might relieve quiescence in CSCs by manipulating notch activity in the niche, as HES1 is a downstream activator of notch.

CONCLUSIONS AND PROJECTIONS

Cancer patients who have been treated and have no evidence of disease are still considered to be at a risk for recurrence for up to a few years, potentially from residual cells which include TICs, which may give rise to metastatic tumors. While a great deal of information is not available on identifying TICs, the scenario to target these cells is very attractive and more effort is needed in characterizing these cells which could give rise to tumors. Depending on cancer type, markers may be more or less unique to the TIC or shared with the mature tumor as in the MUC1 instance. Active immunotherapy is potentially a means to induce long lived protection from a recurrence among cancer patients. Application of some of the aforementioned considerations such as choosing relevant targets, combination therapies to enhance immune efficacy, applying these approaches to a patient population with a low tumor burden or where they can be screened for known precursors of cancer, may help to effectively develop cancer vaccines. Targeting TICs using this approach becomes very attractive towards a promising treatment option for cancer.

LIST OF ABBREVIATIONS

CSCs = Cancer Stem Cells

TIC = Tumor Initiating Cells

REFERENCES

[1] Houghton AN. Cancer antigens: immune recognition of self and altered self. J Exp Med 1994; 180(1): 1-4.

[2] Challis GB, Stam HJ. The spontaneous regression of cancer. A review of cases from 1900 to 1987. Acta Oncol 1990; 29(5): 545-50.

[3] Zhang L, Conejo-Garcia JR, Katsaros D, *et al.* Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003; 348(3): 203-13.

[4] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414(6859): 105-11.

[5] Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. J Exp Med 1996; 183(4): 1797-806.

[6] Goodell MA. Multipotential stem cells and 'side population' cells. Cytotherapy 2002; 4(6): 507-8.

[7] Rajan P, Srinivasan R. Targeting cancer stem cells in cancer prevention and therapy. Stem Cell Rev 2008; 4(3): 211-6.

[8] Adams JM, Strasser A. Is tumor growth sustained by rare cancer stem cells or dominant clones? Cancer Res 2008; 68(11): 4018-21.

[9] Simon RM, Steinberg SM, Hamilton M, *et al*. Clinical trial designs for the early clinical development of therapeutic cancer vaccines. J Clin Oncol 2001; 19(6): 1848-54.

[10] Simon R. Clinical trial designs for therapeutic cancer vaccines. Cancer Treat Res 2005; 123: 339-50.

[11] Adamina M, Oertli D. Antigen specific active immunotherapy: lessons from the first decade. Swiss Med Wkly 2005; 135(15-16): 212-21.

[12] Srinivasan R, Van Epps DE. Specific active immunotherapy of cancer: potential and perspectives. Rev Recent Clin Trials 2006; 1(3): 283-92.

[13] Hoos A, Parmiani G, Hege K, *et al.* A clinical development paradigm for cancer vaccines and related biologics. J Immunother 2007; 30(1): 1-15.

[14] Finke LH, Wentworth K, Blumenstein B, *et al.* Lessons from randomized phase III studies with active cancer immunotherapies--outcomes from the 2006 meeting of the Cancer Vaccine Consortium (CVC). Vaccine 2007; 25 Suppl 2: B97-B109.

[15] Sosman JA, Unger JM, Liu PY, *et al.* Adjuvant immunotherapy of resected, intermediate-thickness, node-negative melanoma with an allogeneic tumor vaccine: impact of HLA class I antigen expression on outcome. J Clin Oncol 2002; 20(8): 2067-75.

[16] Sondak VK, Sosman JA. Results of clinical trials with an allogenic melanoma tumor cell lysate vaccine: Melacine. Semin Cancer Biol 2003; 13(6): 409-15.

[17] Medical News P. Therion Reports Results Of Phase 3 PANVAC-VF Trial And Announces Plans For Company Sale. 2006 [29June2006]; Available from: http://www.medicalnewstoday.com/articles/46137.php.

[18] Giaccone G, Debruyne C, Felip E, *et al.* Phase III study of adjuvant vaccination with Bec2/bacille Calmette-Guerin in responding patients with limited-disease small-cell lung cancer (European Organisation for Research and Treatment of Cancer 08971-08971B; Silva Study). J Clin Oncol 2005; 23(28): 6854-64.

[19] Medical News Today C. Discontinuation of Onercept in Moderate-To-Severe Psoriasis and Canvaxin(reg) in Stage IV Melanoma. 2005 [09 April]; Available from: http://www.medicalnewstoday.com/articles/22446.php.

[20] Find Articles Cs. CancerVax Announces Results of Phase 3 Clinical Trials of Canvaxin(TM) in Patients With Stage III and Stage IV Melanoma. 2006 [cited 2006 March 2006]; Available from: http://findarticles.com/p/articles/mi_pwwi/is_200603/ai_n16114123.

[21] Antigenics O. Antigenics Reports Phase 3 Results for Oncophage in Kidney Cancer. 2006 [24 March 2006]; Available from: http://www.antigenics.com/news/2006/0324.phtml.

[22] Mayordomo J TA, Miles D, FInke L, Jenkins, H. Long term follow up of patients concommitantly treated with hormone therapy in prospective controlled randomized multicenter clinical study comparing STn-KLH vaccine with KLH control in Stage IV breast cancer following first line chemotherapy. J Clin Oncol 2004; 22(14S): 2547.

[23] Genitope M. Genitope Corporation Reports Initial Results of Phase 3 Clinical Trial of MyVax(R) Personalized Immunotherapy. 2007 [Dec 20, 2007]; Available from: http://ir.genitope.com/phoenix.zhtml?c=142124&p=irol-newsArticle&ID=1089374&highlight=.

[24] CellGenesys. Cell Genesys Halts VITAL-2 GVAX Trial in Advanced Prostate Cancer. 2008; Available from: http://phx.corporate-ir.net/phoenix.zhtml?c=98399&p=irol-newsArticle&ID=1191052&highlight=.

[25] Hege KM, Jooss K, Pardoll D. GM-CSF gene-modifed cancer cell immunotherapies: of mice and men. Int Rev Immunol 2006; 25(5-6): 321-52.

[26] CellGenesys. GVAX immunotherapy for prostate cancer Cell Genesys, Inc; 2008; Available from: http://www.cellgenesys.com/clinical-prostate-cancer.shtml.

[27] Nemunaitis J, Dillman RO, Schwarzenberger PO, *et al.* Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. J Clin Oncol 2006; 24(29): 4721-30.

[28] NovaRx. NovaRx Initiates Pivotal Phase III Clinical Trial in Lung Cancer Patients. 2008; Available from: http://www.novarx.com/press-releases.php.

[29] Molldrem J, Dermime S, Parker K, *et al.* Targeted T-cell therapy for human leukemia: cytotoxic T lymphocytes specific for a peptide derived from proteinase 3 preferentially lyse human myeloid leukemia cells. Blood 1996; 88(7): 2450-7.

[30] The Vaccine Company. PR1 Peptide Clinical trials for AML. 2008; Available from: http://www.thevaccinecompany.com/info/default.asp.

[31] Heslop HE, Stevenson FK, Molldrem JJ. Immunotherapy of hematologic malignancy. Hematology Am Soc Hematol Educ Program 2003; 331-49.

[32] Small EJ, Schellhammer PF, Higano CS, *et al.* Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 2006; 24(19): 3089-94.

[33] Dendreon FAR. Dendreon Announces FDA Advisory Committee Reviewed Provenge® for Hormone Refractory Prostate Cancer. 2007 [March 29, 2007]; Available from: http://investor.dendreon.com/newsroom/releasedetail.cfm?ReleaseID=235970&header=News.

[34] Dendreon. Dendreon Announces FDA Confirms Data Required for Provenge® Licensure. 2007 [May 31, 2007]; Available from: http://investor.dendreon.com/newsroom/releasedetail.cfm?ReleaseID=246500&header=News.

[35] Dendreon. Dendreon Expects Interim Data Analysis for Phase 3 PROVENGE IMPACT Trial in October. 2008; Available from: http://investor.dendreon.com/newsroom/releasedetail.cfm?ReleaseID=323910&header=News.

[36] Dendreon BS. CELLULAR, TISSUE AND GENE THERAPIES ADVISORY COMMITTEE SIPULEUCEL-T BRIEFING DOCUMENT BLA STN 125197/0". 2007 [March 29, 2007]; Available from: http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4291b1_01.pdf.

[37] Srinivasan R, Wolchok JD. Tumor antigens for cancer immunotherapy: therapeutic potential of xenogeneic DNA vaccines. J Transl Med 2004; 2(1): 12.

[38] Cheever MA. Twelve immunotherapy drugs that could cure cancers. Immunol Rev 2008; 222: 357-68.

[39] Lapidot T, Sirard C, Vormoor J, *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 1994; 367(6464): 645-8.

[40] Singh SK, Hawkins C, Clarke ID, *et al.* Identification of human brain tumour initiating cells. Nature 2004; 432(7015): 396-401.

[41] Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003; 100(7): 3983-8.

[42] Dalerba P, Dylla SJ, Park IK, *et al.* Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA 2007; 104(24): 10158-63.

[43] O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 2007; 445(7123): 106-10.

[44] Ricci-Vitiani L, Lombardi DG, Pilozzi E, *et al.* Identification and expansion of human colon-cancer-initiating cells. Nature 2007; 445(7123): 111-5.

[45] Hermann PC, Huber SL, Herrler T, *et al.* Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 2007; 1(3): 313-23.

[46] Li C, Heidt DG, Dalerba P, *et al*. Identification of pancreatic cancer stem cells. Cancer Res 2007; 67(3): 1030-7.

[47] Maitland NJ, Collins AT. Prostate cancer stem cells: a new target for therapy. J Clin Oncol 2008; 26(17): 2862-70.

[48] Beier D, Hau P, Proescholdt M, *et al.* CD133(+) and CD133(-) glioblastoma-derived cancer stem cells show differential growth characteristics and molecular profiles. Cancer Res 2007; 67(9): 4010-5.

[49] Shmelkov SV, Butler JM, Hooper AT, *et al*. CD133 expression is not restricted to stem cells, and both CD133+ and CD133- metastatic colon cancer cells initiate tumors. J Clin Invest 2008; 118(6): 2111-20.

[50] Engelmann K, Shen H, Finn OJ. MCF7 side population cells with characteristics of cancer stem/progenitor cells express the tumor antigen MUC1. Cancer Res 2008; 68(7): 2419-26.

[51] Spisek R, Kukreja A, Chen LC, *et al.* Frequent and specific immunity to the embryonal stem cell-associated antigen SOX2 in patients with monoclonal gammopathy. J Exp Med 2007; 204(4): 831-40.

[52] Sang L, Coller HA, Roberts JM. Control of the reversibility of cellular quiescence by the transcriptional repressor HES1. Science 2008; 321(5892): 1095-100.

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