SYMPOSIUM PAPER

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Cancer vaccines as a therapeutic modality: the long trek

Abstract The development of cancer vaccines has been one of the several false dawns in which initial promising Phase I and Phase II clinical data have not been followed up with conclusive Phase III trials. In this review, we describe some of the successes and failures, and review the most likely reasons for Phase III failure, such as protocol changes, which are common between Phase II and III, and poorly defined patient groups. Nevertheless, significant survival results have been reported with autologous vaccines for colorectal, renal and, more recently, prostate cancer. In addition, it is becoming evident that immunotherapy is potentially synergistic with other treatment modalities, such as chemotherapy, which can reduce T-regulatory activity that inhibits the immune response to cancer vaccines. This potential for synergy should allow cancer vaccines to become part of the standard treatment regimen for many common tumours.

Keywords Immunotherapy · Clinical trial · Cancer vaccines

Introduction

The concept that the immune system can be harnessed to reject cancer cells has been speculated upon throughout history. The first scientific documentation of an attempt to induce tumour regression was performed by William Coley, a New York surgeon, at the turn of the last century (1900s) who noticed regression of a cheek sarcoma which recurred following excision and which only regressed when the wound became infected with erysipelas (*Streptococus pyogenes*) [1]. Every time the patient expressed a severe fever the tumour regressed until it disappeared completely. The patient was still in complete remission 7 years later. Suspecting the cause of the regression to be infection he deliberately infected tumours, which eventually proved fatal, with the patient dying of septicaemia. He produced a 'vaccine' containing two killed bacteria, namely *S. pyogenes* and *Serrelia marcescens*, with which he was able to induce a fever without a live infection. His first case was a success; an inoperable abdominal sarcoma involving the bladder and pelvis completely regressed and remained clear for 26 years when the patient died of a heart attack. Coley went on to treat hundreds of patients and was to document crucial characteristics of the response to vaccine and survival. He noted that a 'fever' in response to the vaccine was essential, injections had to be given daily and doses had to be escalated to prevent tolerance. Furthermore, he suggested that the effect was most pronounced when injected into the tumour or metastatic tissue whenever possible.

Beyond Coley's toxins

Although these guidelines were quite specific, other groups claimed that the 'toxin', or Coley's toxin as it became known, was ineffective. Consequently, these findings fell into disrepute and it is only in recent years that a renaissance in immunology has once again brought these studies to the fore. Most particularly, the discovery of the pathogen-associated molecular pattern (PAMP) families [2], typified by Toll-like receptors (TLRs), has shown that interaction of the innate immune system with pathogens can lead to stimulation of the adaptive immune system. Indeed, it is widely believed that such interactions can explain the immunological activity of many modern adjuvants commonly

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used in immunotherapy. It is believed that their mechanism of action is to induce a cytokine cascade after ligation of a TLR. This is exemplified by Bacille Calmette-Guerin (BCG), which is used in the treatment of bladder cancer directly as a live organism and also has been used as an adjuvant.

Vaccines were first used to induce an adaptive immune response at the turn of the last century when large numbers of autologous and allogeneic tumour cells were administered at 14-day intervals. Clinical benefit from this approach was first reported by Coca et al. [3, 4]. Progress from 1970s onwards has been rapid and many tumour vaccines have been developed for cancer, most of them using tumour antigens derived from melanoma (either from cell lines or cell lysate) combined with an immune stimulant, such as BCG.

Correlations between clinical responses, survival and immune responses have been reported using a number of approaches. These include gangliosides supplemented with BCG [5] and allogeneic whole cell vaccines [6], both of which strongly suggest that an effective immune response can target tumour-specific antigens (TSAs). This was first demonstrated in animal models by Globerson and Feldman [7] and Robert Baldwin [8].

However, it is becoming clear that adaptive immune responses to single TSAs may not be completely effective and thus raises the issue of single antigens versus polyvalent vaccines. There is substantial evidence to suggest that an effective immune response can result in downregulation of the antigen by the tumour. The most likely explanation for this is "immune-editing", as described by Dunn et al. [9], which proposes that the genetic instability of tumours may lead to mutations that can avoid immune surveillance. Consequently, in spite of a trend towards reductionism of important dominant single epitopes, most recent trials use several such epitopes simultaneously.

Why do Phase III trials go wrong?

The history of cancer vaccines is one of the encouraging Phase II trials followed by poor Phase III studies. There may be several explanations for the differences between Phase II and Phase III, including differences in manufacture, scale up and lack of control in administration. Phase II trials are often "open label" and have a tight control of patient selection. Phase III tend to be double blinded and have a wide patient population, which is less tightly regulated. Thus, it is common that Phase II patients have been serendipitously selected for a population most likely to respond, without actually knowing the reason. Therefore, in Phase II trials it would be sensible to identify markers which may help in the patient selection of the optimal group in which Phase III studies could be carried out.

The ganglioside vaccine developed by Livingston et al. [5] showed a prolonged disease-free survival when given with BCG versus BCG alone. However, this was only significant when patients with elevated anti-GM2 antibody levels pre-study were excluded. A large randomised study of this vaccine against high-dose interferon failed to show any benefit. Furthermore, analysis of these trials reveals two major changes, the first being that low-dose Cyclophosphamide was used prior to vaccination in the first trial but not in the second and that, perhaps more importantly, the adjuvant had been changed from BCG to QS-21. It is likely that this fundamental change of protocol had direct impact on the function of the vaccine. BCG boosts cell-mediated T_H1 immune responses, characterised by IL-2 and IFNγ, whereas QS-21 induces both a T_H1 and a strong T_H2 response, characterised by IL-4, IL-6 and IL-10. A dominant T_H2 response is usually associated with disease progression.

There are many such examples in a number of clinical settings where Phase III failure following Phase II success has occurred, for instance, pemtumomab antibody for ovarian cancer [10] and Stn-KLH vaccine for breast cancer [11, 12], both failed to show response in Phase III trials. A more recent example is that of the polyvalent whole cell-based vaccine developed by Donald Morton, which demonstrated remarkable 5-year survival figures in resected stage IV melanoma patients in a large single institution study [6]. However, a multi-centre, worldwide randomised study was stopped because the monitoring committee could see no likelihood of a significant divergence of the two arms. Possible reasons for this unexpected failure include the fact that the placebo arm still received non-specific stimulation with BCG. Another possibility is that the single institution study treated a selected population, whereas the multi-centre study had over 80 centres in four continents and may have recruited a more heterogeneous population. It may be important to consider variables, such as life style, diets and supplement, etc., when conducting these studies at major cancer centres are more likely to give advice regarding this aspect of management.

If the Phase II data is real, and not artificially selected, then it is likely that the population does need to be selected, whether for pre-existing immune responses [5] or for having an immune response to treatment before beginning Phase III trials. The importance of such selection has been highlighted by the rapid success and approval of Herceptin, which enhances survival in 30% of breast cancer patients with Her-2/neu expression. If patients are not selected by marker expression then the activity would not have achieved significance across all metastatic patients. A similar scenario will surely need to be considered in the development of novel cancer vaccines.

As discussed previously, the innate immune response has been shown to be important determining the nature of T-helper responses, in particular, T_H1 cell-mediated responses or T_H2 humoral responses. Dogma dictates that the former is most likely to be beneficial in the control of solid tumours. However, it is of great interest that many cancers actively suppress T_H1 responses. This

was clearly demonstrated by the reversal of this suppression upon surgical removal of Duke's A, B and C colon cancers [13]. This suppression is present in many other tumour types and poses a major problem in unresectable disease in that the immune response to a cancer is subject to such immune suppression.

We have previously shown that a heat killed *Mycobacterium vaccae* (SRL-172) can induce a T_H1 response in approximately 30% of advanced melanoma patients and that this appears to correlate with increased survival [14]. More recently we showed that the addition of low-dose IL-2 to SRL-172 can enhance the number of patients who switched to a T_H1 response and that this also correlates with clinical responses [15].

Interestingly, during the Phase I/II trial of *M. vaccae* one of the 'melanoma' patients turned out to be a primary lung cancer case with cervical and supraclavicular secondary metastases. After commencement with *M. vaccae* she had her lymph nodes irradiated because of local discomfort. Even though the primary was not irradiated, there was regression in the primary tumour, suggesting that radiotherapy (RT) had induced an immune response in the presence of *M. vaccae*. Consequently, *M. vaccae* was tested in lung cancer (both small cell and non-small cell) in the hope that there may be synergy with standard chemotherapy regimens. A 29% increase in survival was seen in patients given SRL-172 with chemotherapy, as opposed to chemotherapy alone in patients with non-small cell lung carcinoma [16]. A large multi-centre randomised trial failed to confirm this benefit, although it did detect a significant improvement in quality of life and chemotherapy side effects on the SRL-172 arm [17, 18].

This is, therefore, another example of a Phase III trial not replicating earlier results. However, there were important differences in the conduct of the studies. Only 46% of patients randomised to SRL-172 received the scheduled number of doses in the treatment phase and 63% did not receive any in the follow-up phase. Moreover, less than half the injections were given intradermally, as per protocol, and were more usually given subcutaneously. This fundamental difference may explain why there was no significant immune exposure and hence response.

The lessons from these trials raise two main issues; can protocols be designed in such a way as to minimise variability and can biomarkers be used to identify potentially responding patients?

Proof of principle

In spite of the depressing Phase III results to date it should not be forgotten that there are two significantly positive Phase III randomised cancer vaccine trials; one for renal cancer, and the other for colorectal. Both of these employed autologous vaccines and, hence, are labour intensive procedures as opposed to pharmaceutical vaccine products. However, it is certainly encouraging that such clinical data exists and is highly supportive of continued effort in the development of therapeutic cancer vaccines.

The renal vaccine trial [19] randomised 558 patients at 55 institutions before nephrectomy. A 5-year hazard ratio for progression was 1.58 (95% [11.05–2.37]) in favour of the vaccine group. The colorectal trial [20] consisted of 254 patients with colon cancer prior to surgery being randomised to adjuvant vaccine or no treatment. The 5.3-year median follow-up showed a 44% risk reduction in recurrence in all vaccinated patients. The data was most significant in stage II patients. A review of the major areas of cancer vaccine research is presented in Table 1.

The ideal indication

One method to improve the success of Phase III trials is to ensure that the target patient group is extremely well defined. As we have discussed, even slight variations in clinical presentation can have a devastating effect on trial outcome. Vaccines as adjuvant therapy are most

Table 1 Major cancer vaccine groups with associated clinical experience

Type of therapy	Example	Reference
Non-specific		
Adjuvant therapy	BCG	[15, 34, 35]
	IL2	
	Mycobacterium vaccae	
Immune modulation	Anti-CTLA4	[36]
Specific		
Antibodies	Trastuzumab (Herceptin)	[37–39]
	Cetuximab (Erbitux)	
	Rituximab (Rituxan)	
Anti-idiotype	105AD7	[40]
Recombinant protein	CEA	[41–44]
	MAGE-3	
	NY-ESO-1	
	HPV-fusion	
Recombinant ganglioside	GM2 ganglioside	[5]
Peptide vaccine	MART-1	[45–49]
	Tyrosinase	
	NY-ESO-1	
	K-ras and p53	
	WT1	
Nucleic acids	Gp100 plasmid	[50–52]
	PSMA	
	Allovectin	
Recombinant virus	DISC	[52–54]
	MVA	
	Adenovirus	
Dendritic cells	Protein loaded	[55–59]
	Peptide loaded	
	Lysate loaded	
	Virus infected	
	Transfected	
Whole cell vaccines	Syngeneic colon	[19, 20, 26, 60]
	Syngeneic renal	
	Allogeneic melanoma	
	Allogeneic prostate	

likely to be effective in conditions where there is a high risk of relapse over a given period of time, and, when this does occur, the rate of disease progression should not be too rapid. Most cancer vaccines do not usually induce dramatic tumour reductions. The most likely patients to benefit are those with totally resected disease with a high risk of relapse, such as stage III melanoma or in slowly progressive diseases with minimal tumour volume. The presence of a good surrogate marker would be an additional advantage. Given these constraints, resected renal and colorectal cancers make good candidates. However, as colorectal appears to be more suitable in early disease (Duke's A, B—stage II), these trials, in the absence of good tumour markers will take longer than most pharmaceutical companies, let alone small biotechs, are willing to fund. Although melanoma has been a favourite tumour for cancer vaccine development, there is a strong argument that stage IV disease is an inappropriate indication due to the unpredictable and rapid nature of disease progression.

Prostate cancer vaccines

Perhaps a better example is prostate cancer. This has a more predictable disease progression than most solid tumours, as well as having an excellent biomarker in the form of prostate-specific antigen (PSA) [21]. It should be noted, however, that PSA is not acceptable as a clinical trial endpoint for regulatory approvals [22], most likely because of the lack of prospective or retrospective trials correlating PSA with overall survival. Leaving this aside, treatment can be commenced on the basis of three or more successive rises in PSA levels, the logarithm of which may be used to calculate the PSA velocity (PSAV). Treatment may be commenced when hormone-resistant patients present with a rising PSA as the only evidence of relapse. This group is now eligible for Taxol-based chemotherapy; however, most clinicians agree that a non-toxic approach is more preferable in asymptomatic patients. Indeed, there is even a strong argument that treatment of rising PSA before hormonal therapy could be effective in some patients, thus sparing them months of hormonal therapy and the resultant medical castration.

A number of vaccines are in trial in prostate cancer. Dendreon, which uses autologous dendritic cells (DC), expanded ex vivo and then pulsed with a prostatic acid phosphatase (PAP) fusion protein, has been used as a vaccine. Early studies correlated clinical outcome with immune response [23] and, very recent results, have claimed a survival advantage in a placebo controlled, double blind, randomised study (http://www.dendreon.com). Cellgenesys have employed an allogeneic whole tumour cell transfected with GM-CSF for the same indication in two randomised studies. No results are available at the time of writing.

Onyvax, a private UK company, has developed an allogeneic whole cell vaccine with no genetic modifica-

tion. After pre-clinical proof of concept [24] and a Phase I/II study of several cell combinations [25], a candidate was selected for Phase II studies. The results have recently been published [26] and show that 40% of patients had a prolonged reduction in the rate of rise of PSA as well as a median time to progression of 58 weeks. This is considerably longer than other studies in the same population and randomised studies are now being planned.

A virus vector-based vaccine by Therion has recently demonstrated that patients may benefit from vaccine prior to the development of hormonal resistant disease. Arlen et al. [27] have reported in a randomised study that patients who commenced vaccine 6 months prior to anti-androgen (AA) therapy (which was added in if PSA progression had occurred by 6 months) had a median time to treatment failure of 25.9 months compared to 15.9 months for patients who commenced AA before vaccine was administered [27].

Vaccine plus other modalities

The study of Arlen et al. highlights the potential for vaccines, and other immunotherapies, to be at least additive, if not synergistic, with other modalities, such as RT or chemotherapy. There is a considerable literature on the potential synergy between RT and different immunotherapies including recent reports of synergy with TLR agonists.

Chemotherapy is often thought to be potentially detrimental because of its immunosuppressive properties. However, it is becoming apparent that chemotherapy may actually have some beneficial properties caused by the suppression of regulatory T-cell activity, which in turn depresses the immune response directed against the "self" antigens expressed by the cancer. Interestingly, if these cells are specifically targeted with monoclonal antibodies against CD25 or CTLA-4, significant autoimmunity, such as colitis, has been reported. It is possible that an optimised optimal chemotherapy regimen may be able to suppress this activity without overt toxicity. Furthermore, the cytotoxic activities of many chemotherapeutic agents may enhance vaccination strategies by releasing TSAs from cancer cells and making them more available to DC for antigen presentation.

Biomarkers as predictors of outcome

The second major method that can be employed to improve Phase III outcomes is the increased use of biomarkers. The term 'biomarker' refers to any biological marker that reflects disease status but may not necessarily be involved in the disease process itself. These vary from being highly disease and stage specific, for example, PSA, to non-specific parameters of inflammation that may correlate with an increased risk of a number of

diseases. Examples of these include ischaemic heart disease and cancer which may employ C-reactive protein (CRP) [28] as a marker and is associated with increased coronary artery disease when continually elevated. Furthermore, there are many good cancer biomarker candidates which are regularly used by clinicians but which have not necessarily been validated for clinical trial use. Well-known examples include α-feto protein (αFP) and human chorionic gonadotropin (HCG) in chorionic and testicular cancers [29], CA125 in ovarian cancers [30], CA19.9 in pancreatic cancer [31], carcinoma embryonic antigen (CEA) in colorectal cancer [32] and PSA in prostate cancers [21]. Perhaps the most indicative of all of the cancer biomarkers is when tumours are caused by translations or fused genes, such as those seen in chronic myeloid leukaemia. Polymerase chain reaction (PCR) can be used to identify highly specific biomarkers, with PCR transcript negativity confirming total disease elimination [33].

Numbers of potential biomarkers are increasing exponentially, particularly in the post-genomic or proteomic era. The situation has become further complicated by recent trends to use immune response biomarkers which may have utility in identifying patients who are mounting a meaningful clinical response at an early stage. Clearly, it would be advantageous to pick out these patients as early as possible and also, to move non-responding patients onto alternative treatment modalities. Furthermore, accurate biomarkers could greatly reduce the time needed to assess if a drug has significant clinical activity in Phase II trials before progressing to larger, and more costly, Phase III trials. Current clinical endpoints, as we have discussed, can lead to the development of long, and prohibitively expensive, trials. The benefit to patients is obvious, since clinically efficacious therapies could be identified at an early stage, thus greatly improving survival since the immune suppression that accompanies advanced disease would be avoided.

Summary: the long trek

Having commenced at least over a century ago, the quest for a registered cancer vaccine has indeed been a "long trek". However, the success of randomized autologous studies, together with very encouraging Phase II studies in a variety of tumour types, has given new grounds for optimism. Defined patient groups and the ability to combine with other treatment modalities will lead to greater clinical effectiveness. The use of biomarkers will greatly help to achieve this goal, if used correctly.

Historically, most biomarkers in clinical use are defined as either being related to the disease process, for example PSA, or as being involved in the putative mechanism of vaccine efficacy. Particularly in the latter case, the evidence for one particular immunological mechanism being the sole effector function is variable. Clearly, T-cell immunity is important, however, it remains unclear if this is the only mechanism at play. Indeed, it seems unlikely that this is so given the variable MHC expression of tumours and their varying degrees of immunogenicity.

Therefore, we propose a more empirical approach, in which no predictions are made as to the nature of the immune response generated and multiple parameters are recorded. In this way, "immunological profiles" can be built up and then correlated with either clinical outcome or more conventional biomarkers. Hence, the use of large numbers of biomarkers becomes one of quantity rather than quality. It is still likely that many of these analytes are indeed involved in the immune response, but by removing the relatively narrow range of parameters

Table 2 Over 40 different parameters were recorded at each clinical visit for patients on the Onyvax phase II trial. When combined, these produced a database which contained 20,000 variables which was then subjected to artificial neural network analysis to identify patterns associated with clinical responses

Rank	Sensitivity analysis	
	Cohort 1	Cohort 2
1	IFNγ (PCR transcript)	IL10 (protein)
2	IFNγ (protein)	IL2 (protein)
3	CD4 proliferation to LnCaP	IL10 (protein)
4	CD8$^+$ CD38$^+$ (FACS)	CD3$^+$ CD28$^-$ (FACS)
5	Time in days	CD62L$^+$ CD28$^-$ (FACS)
6	IL10 (protein)	CD4 proliferation to LnCaP
7	CD8 proliferation to PHA	CD8$^+$ CD38$^-$ (FACS)
8	CD62L$^+$ CD28$^-$ (FACS)	CD8 proliferation to PHA
9	CD4 proliferation to PHA	TNFα (protein)
10	CD4$^+$ CD38$^+$ (FACS)	CD8 proliferation to LnCaP
11	IL10 (PCR transcript)	IFNγ (PCR transcript)

Variables were then ranked in order of importance using sensitivity analysis for Cohort 1 (non-metastatic) and Cohort 2 (metastatic). Clear differences in the nature of the immune response mounted by both groups can be seen, with cohort 1 being more "TH1-like" and cohort 2 showing a "T$_H$2-like" profile. These data demonstrate that, despite the fact that both groups contained clinical responders, the nature of the immunological response was different between them

normally collected, we ensure that almost all immunological mechanisms are measured. It is unlikely that any one single analyte will show perfect correlation with clinical response, but, when used in combination with a large number of variables, patterns begin to emerge.

A good example of how immunological parameters may be employed as biomarkers comes from the Onyvax whole cell vaccine Phase II trial. A large amount of data was collected using a variety of multi-parametric techniques. Both PSA and time to disease progression are useful clinical indicators, and both of these were correlated with each recorded variable in turn. No significant trends could be discovered. Therefore, artificial neural network (ANN) analysis was employed and clear discrimination between responding and non-responding patients was achieved (Table 2). These patterns were not immediately apparent using classical statistical analysis [26]. A further advantage of this approach is that ANNs may be used predictively by assessing the patient's "immune profile" to conduct a risk assessment for the patient before any treatment is administered. This may be precisely what is required to increase the efficiency of patient group selection before the onset of advanced clinical trials.

Future chances of success will be enhanced by having an optimal vaccine candidate, in the optimal patient population, which has been screened with the optimal set of biomarkers. Antigen presentation can be enhanced by a number of new approaches including adjuvants and TLR agonists. The immune response can be increased using treatments known to reduce regulatory T-cell activity. These include some chemotherapeutic agents and antibodies to CTLA-4 and IL2. Most importantly, immunotherapies, including cancer vaccines, would appear to work better, or in synergy with, other treatments. It is thus now more likely that a cancer vaccine will become part of the standard treatment for cancer.

References

1. Coley WB (1991) The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res 262:3
2. Medzhitov R, Janeway CA Jr (2002) Decoding the patterns of self and nonself by the innate immune system. Science 296:298
3. Coca A, Dorrance G, Lebredo M (1912) Vaccination in cancer: a report of the results of vaccination therapy as applied to seventy-nine cases of human cancer. J Immunol Exp Ther 13:543
4. Coca A, Gilman G (1909) The specific treatment of carcinoma. Philos J Sci Med 4:381
5. Livingston PO, Wong GY, Adluri S, Tao Y, Padavan M, Parente R, Hanlon C, Calves MJ, Helling F, Ritter G et al (1994) Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J Clin Oncol 12:1036
6. Morton DL, Hsueh EC, Essner R, Foshag LJ, O'Day SJ, Bilchik A, Gupta RK, Hoon DS, Ravindranath M, Nizze JA, Gammon G, Wanek LA, Wang HJ, Elashoff RM (2002) Prolonged survival of patients receiving active immunotherapy with Canvaxin therapeutic polyvalent vaccine after complete resection of melanoma metastatic to regional lymph nodes. Ann Surg 236:438
7. Globerson A, Feldman M (1964) Antigenic specificity of Benzo(a)pyrene-induced sarcomas. J Natl Cancer Inst 32:1229
8. Baldwin RW (1973) Immunological aspects of chemical carcinogenesis. Adv Cancer Res 18:1
9. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991
10. Pavlou AK, Belsey MJ (2005) The therapeutic antibodies market to 2008. Eur J Pharm Biopharm 59:389
11. Adis International Ltd. (2003) Cancer vaccine THERATOPE-Biomira. Drugs R D 4:236
12. Braun DP, Crist KA, Shaheen F, Staren ED, Andrews S, Parker J (2005) Aromatase inhibitors increase the sensitivity of human tumor cells to monocyte-mediated, antibody-dependent cellular cytotoxicity. Am J Surg 190:570
13. Heriot AG, Marriott JB, Cookson S, Kumar D, Dalgleish AG (2000) Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. Br J Cancer 82:1009
14. Maraveyas A, Baban B, Kennard D, Rook GA, Westby M, Grange JM, Lydyard P, Stanford JL, Jones M, Selby P, Dalgleish AG (1999) Possible improved survival of patients with stage IV AJCC melanoma receiving SRL 172 immunotherapy: correlation with induction of increased levels of intracellular interleukin-2 in peripheral blood lymphocytes. Ann Oncol 10:817
15. Nicholson S, Guile K, John J, Clarke IA, Diffley J, Donnellan P, Michael A, Szlosarek P, Dalgleish AG (2003) A randomized phase II trial of SRL172 (Mycobacterium vaccae) +/− low-dose interleukin-2 in the treatment of metastatic malignant melanoma. Melanoma Res 13:389
16. Assersohn L, Souberbielle BE, O'Brien ME, Archer CD, Mendes R, Bass R, Bromelow KV, Palmer RD, Bouilloux E, Kennard DA, Smith IE (2002) A randomized pilot study of SRL172 (Mycobacterium vaccae) in patients with small cell lung cancer (SCLC) treated with chemotherapy. Clin Oncol (R Coll Radiol) 14:23
17. Harper-Wynne CL, Sumpter K, Ryan C, Priest K, Norton A, Ross P, Ford HE, Johnson P, O'Brien ME (2005) Addition of SRL 172 to standard chemotherapy in small cell lung cancer (SCLC) improves symptom control. Lung Cancer 47:289
18. O'Brien ME, Anderson H, Kaukel E, O'Byrne K, Pawlicki M, Von Pawel J, Reck M (2004) SRL172 (killed Mycobacterium vaccae) in addition to standard chemotherapy improves quality of life without affecting survival, in patients with advanced non-small-cell lung cancer: phase III results. Ann Oncol 15:906
19. Jocham D, Richter A, Hoffmann L, Iwig K, Fahlenkamp D, Zakrzewski G, Schmitt E, Dannenberg T, Lehmacher W, von Wietersheim J, Doehn C (2004) Adjuvant autologous renal tumour cell vaccine and risk of tumour progression in patients with renal-cell carcinoma after radical nephrectomy: phase III, randomised controlled trial. Lancet 363:594
20. Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG Jr, Pinedo HM (1999) Active specific immunotherapy for stage II and stage III human colon cancer: a randomised trial. Lancet 353:345
21. Small EJ, Roach M (2002) Prostate-specific antigen in prostate cancer: a case study in the development of a tumor marker to monitor recurrence and assess response. Semin Oncol 29:264
22. FDA (2004) FDA public workshop on clinical trial endpoints in prostate cancer
23. Rini BI, Small EJ (2003) The potential for prostate cancer immunotherapy. Crit Rev Oncol Hematol 46(Suppl):S117
24. Hrouda D, Souberbielle BE, Kayaga J, Corbishley CM, Kirby RS, Dalgleish AG (1998) Mycobacterium vaccae (SRL172): a potential immunological adjuvant evaluated in rat prostate cancer. Br J Urol 82:870
25. Eaton JD, Perry MJ, Nicholson S, Guckian M, Russell N, Whelan M, Kirby RS (2002) Allogeneic whole-cell vaccine: a phase I/II study in men with hormone-refractory prostate cancer. BJU Int 89:19

26. Michael A, Ball G, Quatan N, Wushishi F, Russell N, Whelan J, Chakraborty P, Leader D, Whelan M, Pandha H (2005) Delayed disease progression after allogeneic cell vaccination in hormone-resistant prostate cancer and correlation with immunologic variables. Clin Cancer Res 11:4469

27. Arlen PM, Gulley JL, Todd N, Lieberman R, Steinberg SM, Morin S, Bastian A, Marte J, Tsang KY, Beetham P, Grosenbach DW, Schlom J, Dahut W (2005) Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer. J Urol 174:539

28. Koenig W (2005) Predicting risk and treatment benefit in atherosclerosis: the role of C-reactive protein. Int J Cardiol 98:199

29. Segal R, Lukka H, Klotz LH, Eady A, Bestic N, Johnston M (2001) Surveillance programs for early stage non-seminomatous testicular cancer: a practice guideline. Can J Urol 8:1184

30. Lewis S, Menon U (2003) Screening for ovarian cancer. Expert Rev Anticancer Ther 3:55

31. Ko AH, Hwang J, Venook AP, Abbruzzese JL, Bergsland EK, Tempero MA (2005) Serum CA19-9 response as a surrogate for clinical outcome in patients receiving fixed-dose rate gemcitabine for advanced pancreatic cancer. Br J Cancer 93:195

32. Bendardaf R, Lamlum H, Pyrhonen S (2004) Prognostic and predictive molecular markers in colorectal carcinoma. Anticancer Res 24:2519

33. Cilloni D, Saglio G (2004) WT1 as a universal marker for minimal residual disease detection and quantification in myeloid leukemias and in myelodysplastic syndrome. Acta Haematol 112:79

34. Totterman TH, Loskog A, Essand M (2005) The immunotherapy of prostate and bladder cancer. BJU Int 96:728

35. de Gast GC, Batchelor D, Kersten MJ, Vyth-Dreese FA, Sein J, van de KaW, Nooijen WJ, Nieweg OE, de Waal MA, Boogerd W (2003) Temozolomide followed by combined immunotherapy with GM-CSF, low-dose IL2 and IFN α in patients with metastatic melanoma. Br J Cancer 88:175

36. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, Sherry RM, Topalian SL, Kammula US, Royal RE, Restifo NP, Haworth LR, Levy C, Mavroukakis SA, Nichol G, Yellin MJ, Rosenberg SA (2005) Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 23:6043

37. Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G (2005) Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 353:1673

38. Goldberg RM (2005) Cetuximab. Nat Rev Drug Discov (Suppl):S10

39. Hennessy BT, Hanrahan EO, Daly PA (2004) Non-Hodgkin lymphoma: an update. Lancet Oncol 5:341

40. Maxwell-Armstrong CA, Durrant LG, Buckley TJ, Scholefield JH, Robins RA, Fielding K, Monson JR, Guillou P, Calvert H, Carmichael J, Hardcastle JD (2001) Randomized double-blind phase II survival study comparing immunization with the anti-idiotypic monoclonal antibody 105AD7 against placebo in advanced colorectal cancer. Br J Cancer 84:1443

41. Samanci A, Yi Q, Fagerberg J, Strigard K, Smith G, Ruden U, Wahren B, Mellstedt H (1998) Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. Cancer Immunol Immunother 47:131

42. Vantomme V, Dantinne C, Amrani N, Permanne P, Gheysen D, Bruck C, Stoter G, Britten CM, Keilholz U, Lamers CH, Marchand M, Delire M, Gueguen M (2004) Immunologic analysis of a phase I/II study of vaccination with MAGE-3 protein combined with the AS02B adjuvant in patients with MAGE-3-positive tumors. J Immunother 27:124

43. Davis ID, Chen W, Jackson H, Parente P, Shackleton M, Hopkins W, Chen Q, Dimopoulos N, Luke T, Murphy R, Scott AM, Maraskovsky E, McArthur G, MacGregor D, Sturrock S, Tai TY, Green S, Cuthbertson A, Maher D (2004) Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. Proc Natl Acad Sci USA 101:10697

44. Hallez S, Simon P, Maudoux F, Doyen J, Noel JC, Beliard A, Capelle X, Buxant F, Fayt I, Lagrost AC, Hubert P, Gerday C, Burny A, Boniver J, Foidart JM, Delvenne P, Jacobs N (2004) Phase I/II trial of immunogenicity of a human papillomavirus (HPV) type 16 E7 protein-based vaccine in women with oncogenic HPV-positive cervical intraepithelial neoplasia. Cancer Immunol Immunother 53:642

45. Speiser DE, Lienard D, Rufer N, Rubio-Godoy V, Rimoldi D, Lejeune F, Krieg AM, Cerottini JC, Romero P (2005) Rapid and strong human CD8+ T cell responses to vaccination with peptide, IFA, and CpG oligodeoxynucleotide 7909. J Clin Invest 115:739

46. Slingluff CL Jr, Petroni GR, Yamshchikov GV, Hibbitts S, Grosh WW, Chianese-Bullock KA, Bissonette EA, Barnd DL, Deacon DH, Patterson JW, Parekh J, Neese PY, Woodson EM, Wiernasz CJ, Merrill P (2004) Immunologic and clinical outcomes of vaccination with a multiepitope melanoma peptide vaccine plus low-dose interleukin-2 administered either concurrently or on a delayed schedule. J Clin Oncol 22:4474

47. Khong HT, Yang JC, Topalian SL, Sherry RM, Mavroukakis SA, White DE, Rosenberg SA (2004) Immunization of HLA-A*0201 and/or HLA-DPbeta1*04 patients with metastatic melanoma using epitopes from the NY-ESO-1 antigen. J Immunother 27:472

48. Carbone DP, Ciernik IF, Kelley MJ, Smith MC, Nadaf S, Kavanaugh D, Maher VE, Stipanov M, Contois D, Johnson BE, Pendleton CD, Seifert B, Carter C, Read EJ, Greenblatt J, Top LE, Kelsey MI, Minna JD, Berzofsky JA (2005) Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol 23:5099

49. Oka Y, Tsuboi A, Taguchi T, Osaki T, Kyo T, Nakajima H, Elisseeva OA, Oji Y, Kawakami M, Ikegame K, Hosen N, Yoshihara S, Wu F, Fujiki F, Murakami M, Masuda T, Nishida S, Shirakata T, Nakatsuka S, Sasaki A et al. (2004) Induction of WT1 (Wilms' tumor gene)-specific cytotoxic T lymphocytes by WT1 peptide vaccine and the resultant cancer regression. Proc Natl Acad Sci USA 101:13885

50. Rosenberg SA, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer LJ, Morton KE, Mavroukakis SA, White DE (2003) Inability to immunize patients with metastatic melanoma using plasmid DNA encoding the gp100 melanoma-melanocyte antigen. Hum Gene Ther 14:709

51. Mincheff M, Tchakarov S, Zoubak S, Loukinov D, Botev C, Altankova I, Georgiev G, Petrov S, Meryman HT (2000) Naked DNA and adenoviral immunizations for immunotherapy of prostate cancer: a phase I/II clinical trial. Eur Urol 38:208

52. Galanis E (2002) Technology evaluation: Allovectin-7, Vical. Curr Opin Mol Ther 4:80

53. Rees RC, McArdle S, Mian S, Li G, Ahmad M, Parkinson R, Ali SA (2002) Disabled infectious single cycle-herpes simplex virus (DISC-HSV) as a vector for immunogene therapy of cancer. Curr Opin Mol Ther 4:49

54. Smith CL, Dunbar PR, Mirza F, Palmowski MJ, Shepherd D, Gilbert SC, Coulie P, Schneider J, Hoffman E, Hawkins R, Harris AL, Cerundolo V (2005) Recombinant modified vaccinia Ankara primes functionally activated CTL specific for a melanoma tumor antigen epitope in melanoma patients with a high risk of disease recurrence. Int J Cancer 113:259

55. Barrou B, Benoit G, Ouldkaci M, Cussenot O, Salcedo M, Agrawal S, Massicard S, Bercovici N, Ericson ML, Thiounn N (2004) Vaccination of prostatectomized prostate cancer patients

in biochemical relapse, with autologous dendritic cells pulsed with recombinant human PSA. Cancer Immunol Immunother 53:453

56. Banchereau J, Ueno H, Dhodapkar M, Connolly J, Finholt JP, Klechevsky E, Blanck JP, Johnston DA, Palucka AK, Fay J (2005) Immune and clinical outcomes in patients with stage IV melanoma vaccinated with peptide-pulsed dendritic cells derived from CD34+ progenitors and activated with type I interferon. J Immunother 28:505

57. Pandha HS, John RJ, Hutchinson J, James N, Whelan M, Corbishley C, Dalgleish AG (2004) Dendritic cell immunotherapy for urological cancers using cryopreserved allogeneic tumour lysate-pulsed cells: a phase I/II study. BJU Int 94:412

58. Morse MA, Clay TM, Hobeika AC, Osada T, Khan S, Chui S, Niedzwiecki D, Panicali D, Schlom J, Lyerly HK (2005) Phase I study of immunization with dendritic cells modified with fowlpox encoding carcinoembryonic antigen and costimulatory molecules. Clin Cancer Res 11:3017

59. Gilboa E, Vieweg J (2004) Cancer immunotherapy with mRNA-transfected dendritic cells. Immunol Rev 199:251

60. Hsueh EC, Morton DL (2003) Antigen-based immunotherapy of melanoma: Canvaxin therapeutic polyvalent cancer vaccine. Semin Cancer Biol 13:401