MINIREVIEW

Vaccines based on whole recombinant *Saccharomyces cerevisiae* cells

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Abstract

The ultimate goal of therapeutic vaccines is to activate and exploit the patient's own immune system to vigorously and dynamically seek and eradicate established malignant or virally infected cells. Therapeutic vaccines also offer the potential for preventing disease recurrence. *Saccharomyces cerevisiae*-based vaccines, where the yeast is engineered to express viral or tumor antigens, represent an ideal therapeutic approach due to their ability to stimulate tumor- or viral-specific $CD4^+$ and $CD8^+$ T-cell responses that are capable of reducing disease burden. This review describes preclinical and clinical studies supporting the development of *S. cerevisiae*-based therapeutic vaccines for the treatment of cancer and viral diseases, as well as multimodal strategies in which therapeutic vaccines are combined with cytotoxic drugs to achieve a greater clinical response.

Introduction

Saccharomyces cerevisiae, commonly known as baker's yeast, is a nonpathogenic yeast strain mainly used in the making of beer and bread. It is the first eukaryotic organism whose genome was sequenced and has since become a preclinical model and valuable tool for unraveling the fundamental cellular processes in higher eukaryotes (Galao *et al.*, 2007). *Saccharomyces cerevisiae* is an effective vector in therapeutic vaccines. Stubbs *et al.* (2001) have demonstrated that whole recombinant *S. cerevisiae* expressing foreign antigens can activate dendritic cells (DCs), elicit robust antigen-specific cytotoxic T lymphocyte (CTL) responses, and confer protective cell-mediated immunity against tumor challenge in mice (Stubbs *et al.*, 2001). Lu *et al.* (2004) demonstrated that a yeast-based vaccine can generate antigen-specific immune responses independent of the viability of the yeast itself. Additionally, it has been demonstrated that heat-killed and live yeast elicit equivalent protective immunity (Franzusoff *et al.*, 2005). These findings, and further studies performed by our laboratory and others, make heat-killed *S. cerevisiae* an attractive vaccine vehicle, offering many key benefits such as: (1) the ability to express one or more antigens; (2) cost-effectiveness in large-scale manufacturing; (3) expression of cell-surface ligands ('danger signals') that lead to DC maturation without the need for additional adjuvants; (4) efficient antigen presentation via major histocompatibility complex (MHC) class I and class II pathways, generating antigen-specific T-cell immune responses; (5) lack of yeast-induced host-neutralizing immune responses, allowing multiple vaccinations; and (6) the ability to mount immune responses and protective immunity similar to those of live yeast, eliminating the potential safety risks associated with the use of live cells, especially in immunocompromised patients (Franzusoff *et al.*, 2005; Munson *et al.*, 2008). Here, we review multiple preclinical and clinical studies supporting the use of heat-killed whole recombinant *S. cerevisiae* (hereafter referred to simply as yeast) as a therapeutic vaccine to treat cancer and infectious diseases.

Therapeutic vaccines

The goal of prophylactic vaccines is to prevent infectious diseases by activating humoral immune responses and subsequently producing neutralizing antibodies capable of blocking pathogens from infecting host cells. In contrast,

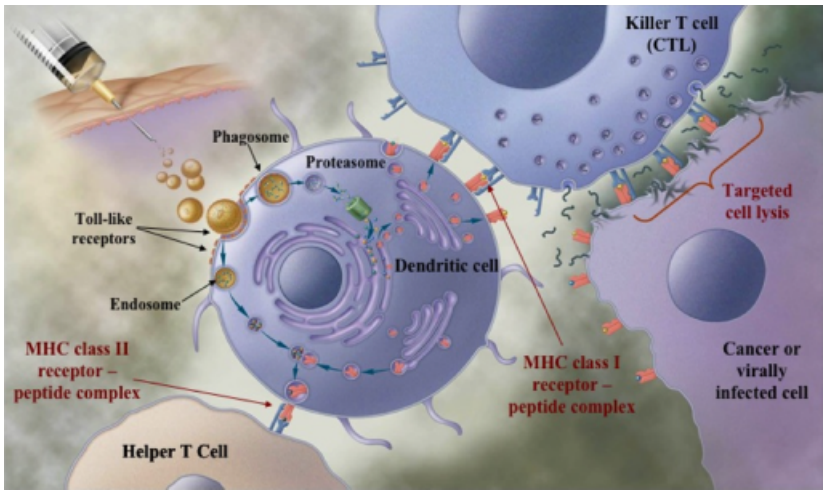

Fig. 1. Proposed mechanism of action of the yeast-CEA vaccine. After injection, yeast-CEA is avidly taken up by DCs and macrophages, driven by the immunogenicity of yeast cell-wall components that transmit 'danger signals' normally associated with microbial infection. The DCs efficiently process antigens to MHC class I and class II pathways through cross-presentation and initiate T cells involved in cell-mediated cytotoxicity. Adapted from GlobImmune, Inc., Louisville, CO.

therapeutic vaccines seek to eliminate abnormal cells (such as virally infected or malignant cells) by generating T-cell-mediated immunity. Elimination of established abnormal cells via a therapeutic vaccine is largely dependent on cell-mediated cytotoxicity executed by $CD8^+$ CTLs. Ideally, however, a therapeutic vaccine must also be able to induce $CD4^+$ T helper responses, because $CD4^+$ T cells can release numerous immunomodulatory cytokines to further drive the generation and proliferation of the robust $CD8^+$ CTL responses essential to the efficacy of a therapeutic vaccine.

Although it is nonpathogenic, yeast has been shown to induce immunologic responses in mammals and is avidly taken up by DCs and macrophages (Fig. 1) (Stubbs *et al.*, 2001; Heintel *et al.*, 2003). The phagocytosis of yeast by DCs is driven by the immunogenicity of yeast cell-wall components, such as β-1,3-D-glucan and mannan, that can transmit 'danger signals' normally associated with microbial infection. These components have strong adjuvant properties and can be detected by pattern recognition receptors such as Toll-like receptors and mannan receptors on DCs (Munson *et al.*, 2008). DCs are the most efficient antigen-presenting cells (APCs). Their unique ability to efficiently process antigens to MHC class I and class II pathways through cross-presentation makes them crucial for initiating both humoral responses and cell-mediated cytotoxicity. Therefore, once inside the host, yeast expressing viral or tumor antigen is easily recognized and subjected to receptor-mediated phagocytosis by DCs for presentation to both MHC class I and class II pathways.

Yeast expressing tumor or viral antigens can be degraded in proteasomes, presented through MHC class I, and recognized by $CD8^+$ CTLs. This subsequently induces the proliferation, maturation, and activation of antigen-specific $CD8^+$ CTLs. Yeast can also be degraded in endosomes, presented to MHC class II, and recognized by $CD4^+$ T helper cells. Engagement of the T-cell receptor and the peptide–MHC complex is the first signal necessary to activate T-cell immunity. The second signal involves the interaction of DC costimulatory molecules with their ligands expressed on the T cell. Yeast vaccine enhances both signals, as increased expression of both MHC class I and class II molecules and increased expression of costimulatory molecules on DCs have been observed (Bernstein *et al.*, 2008; Remondo *et al.*, 2009). In sum, the use of yeast-based vaccines leads to the recruitment and activation of antigen-specific $CD4^+$ and $CD8^+$ T cells (Stubbs *et al.*, 2001; Franzusoff *et al.*, 2005; Munson *et al.*, 2008).

The activation of $CD4^+$ and $CD8^+$ T cells is required to induce the therapeutic immune responses needed to treat malignant or virally infected cells. Specifically, $CD4^+$ T cells release immunostimulatory Th-1 type inflammatory cytokines, such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α), that further induce the activation and proliferation of $CD8^+$ CTLs. These $CD8^+$ CTLs kill abnormal cells via two main mechanisms of action. The first is the release of the cytotoxins perforin and granzymes. Perforin forms holes in the target cell's plasma membrane, allowing granzymes to enter and kill the target cell. Granzymes activate caspase enzymes and induce the production of reactive oxygen species, both of which lead to cell death. Secondly, $CD8^+$ CTLs kill target cells through the interaction of surface protein Fas ligands on activated CTLs and Fas receptors on target cells, which also induces apoptotic cell death (Stenger *et al.*, 1998). Yeast is thus able to activate and inducing maturation of DCs, leading to the generation of antigen-specific $CD4^+$ and $CD8^+$ T-cell responses capable of killing virally infected or malignant cells.

Yeast-based vaccines for cancer immunotherapy

For a therapeutic yeast-based vaccine to effectively generate the $CD8^+$ CTLs necessary to recognize and kill malignant

cells, the yeast must be engineered to express the tumor-specific or tumor-associated antigens selectively expressed or overexpressed on malignant cells. Numerous tumor antigens are currently being investigated in preclinical and clinical studies. However, the yeast-based cancer vaccines reviewed here target two main tumor antigens: yeast-ras targets *ras* oncogenes and yeast-carcinoembryonic antigen (CEA) targets oncofetal CEA.

Yeast-ras

Ras, a family of genes that activates the signaling pathway for cell proliferation, acts downstream of receptor tyrosine kinases such as epidermal growth factor receptor (Lu *et al.*, 2004). Ras activation leads to cell proliferation, differentiation, and survival. Mutations in the ras proto-oncogene family, such as K-, H-, or N-ras, are common and are consistently expressed in many types of solid tumors, including pancreatic (90–100%), colorectal (30–50%), ovarian (20–25%), melanoma (50%), and nonsmall-cell lung (20–30%) cancers (Franzusoff *et al.*, 2005).

In 2004, Lu and colleagues generated whole recombinant yeast-ras vaccines expressing mammalian mutant K-ras proteins and tested their ability to generate the immune responses required for tumor killing in carcinogen-induced lung tumors in mice. Mice exposed to urethane, a chemical carcinogen, develop single amino acid mutations in codon 61 in the ras oncoprotein. Following urethane exposure, lung hyperplasias occur within 2 weeks, adenomas occur in approximately 5 weeks, adenocarcinomas by 16 weeks, and death from respiratory failure within 12 months (Forkert *et al.*, 1992; Horio *et al.*, 1996; Lu *et al.*, 2004). Studies have been conducted with yeast vaccines expressing different mammalian ras proteins, representing some of the most frequent mutations responsible for the constitutive activation of ras oncoprotein. The use of these vaccines to immunize mice in a carcinogen-induced lung tumor model led to two important findings (Bos, 1989; Lu *et al.*, 2004): (1) yeast-ras vaccines can generate regression of established ras mutation-bearing lung tumors in a dose-dependent and antigen-specific manner and (2) dosing regimens that include multiple boosts lead to optimum tumor killing (Lu *et al.*, 2004; Franzusoff *et al.*, 2005). The safety of the yeast-ras vaccine was further evaluated in five preclinical toxicity studies in a rabbit model. Rabbits were injected weekly with 0.5–100 yeast units (YU) for up to 13 weeks. Histopathologic analyses revealed no major side effects in the rabbits. Increased levels of circulatory neutrophils were observed, along with minor injection-site reactions that resolved on their own after 2 weeks (Munson *et al.*, 2008).

These preclinical findings led to the initiation of an open-label, dose-escalation, phase I clinical trial of monotherapy with yeast-ras. The trial enrolled 33 patients with advanced ras mutation$^+$ pancreatic, colorectal, and nonsmall-cell lung cancer (NSCLC). All patients underwent ras genotyping to match each patient's individual mutation with the appropriate yeast-ras vaccine. Most patients had metastatic disease at the time of enrollment and had received an average of three previous therapy regimens before participating in this phase I trial. Subjects received 0.1, 1, 5, 10, 20, or 40 YU of the mutation-matched yeast-ras vaccine (Q61R+Q61L+**G12V** or Q61R+Q61L+**G12C** or Q61R+Q61L+**G12D**), administered subcutaneously for 5 weeks. The overall safety, injection-site reactions, and antigen-specific immune responses were monitored. After 5 weeks of yeast-ras vaccine therapy, no dose-limiting toxicities, therapy-related serious adverse events, or clinically significant laboratory abnormalities were observed at any of the dose levels tested. Approximately 90% of subjects exhibited ras-specific T-cell responses, as demonstrated by lymphocyte proliferation and/or intracellular cytokine staining assays (Franzusoff *et al.*, 2005; Munson *et al.*, 2008).

Yeast-CEA

Oncofetal CEA, the first cell-surface tumor-associated antigen to be described (Gold & Freedman, 1965; Huang & Kaufman, 2002), is a 180-kDa glycoprotein normally expressed in limited areas of the adult human body. However, CEA is overexpressed in nearly 50% of all human tumor types and 80–90% of most colorectal cancers. In cancer patients, significantly elevated cell-surface expression of CEA is associated with more advanced disease and with increased rates of recurrence compared with patients with lower levels of CEA expression. Additionally, upon transformation of epithelial cells, CEA can lose its apical polarity on the cell surface and thus be secreted into the capillaries. It can then be used as a serologic circulating tumor marker in certain cancers (Huang & Kaufman, 2002).

CEA is an attractive target for immunotherapy because it is expressed minimally in normal tissues, but overexpressed in a wide variety of malignant epithelial tissues. Our laboratory has recently developed a recombinant yeast vaccine expressing human CEA antigen (yeast-CEA) (Bernstein *et al.*, 2008). Preclinical studies using chicken ovalbumin antigen demonstrated that recombinant yeast could induce the activation and maturation of DCs *in vitro* and elicit immune and antitumor responses in mice (Stubbs *et al.*, 2001; Stubbs & Wilson, 2002; Lu *et al.*, 2004; Franzusoff *et al.*, 2005). Our laboratory extended these findings by further elucidating the potential mechanisms for yeast-induced tumor-specific immune responses. First, to assess the cellular changes in draining lymph nodes, tumor-free wild-type C57BL/6 mice were administered a single subcutaneous injection of either phosphate-buffered saline (PBS) or 1 YU of yeast-CEA and sacrificed at selected time points. At 2 days postvaccination, the total number of

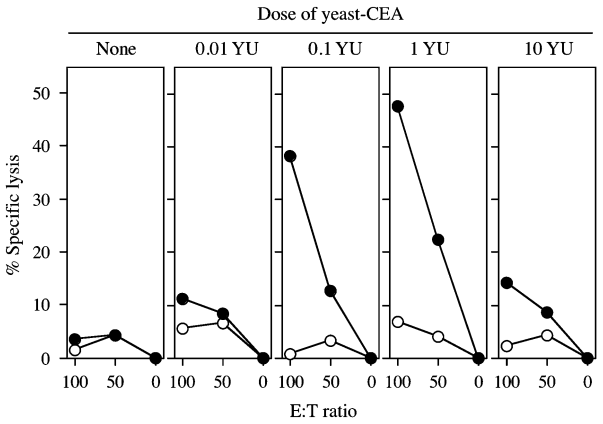

Fig. 2. $CD8^+$ CTL responses after dose escalation of yeast-CEA. CEA-Tg mice were vaccinated with 0, 0.01, 0.1, 1, or 10 YU of yeast-CEA twice at 7-day intervals. Fourteen days after the last vaccination, mice were sacrificed, spleens were harvested, and splenocytes were stimulated with the CEA peptide for 6 days. Lymphocytes were incubated for 5 h with ^{51}Cr-labeled target EL-4 cells pulsed with CEA or VSV-NP control peptide. Radioactivity in the supernatant was measured and specific lysis was calculated. Open circles, EL-4 cells pulsed with the VSV-NP peptide; filled circles, EL-4 cells pulsed with the CEA peptide (Wansley *et al.*, 2008).

lymphocytes and DCs at the draining lymph node doubled compared with mice receiving PBS (Table 1A). Increased levels of activated and mature DCs at the draining lymph nodes can potentiate more CEA antigen cross-presentation to both MHC class I and class II molecules, therefore enhancing populations of activated $CD4^+$ and $CD8^+$ T cells. *In vitro* treatment of DCs with yeast-CEA also significantly increased the expression of multiple costimulatory molecules and the production of various inflammatory cytokines compared with mock-treated DCs (Table 1A). Increased levels of costimulatory molecules such as B7.1 and LFA-3 are highly advantageous because they strengthen the signals required for efficient T-cell activation while enhancing T-cell avidity. Furthermore, the interactions of these costimulatory molecules result in the upregulation of T-cell function and more potent $CD8^+$ CTLs. A plethora of Th-1 and Th-2 cytokines are also upregulated, which enhances the APC and T-cell function. Specifically, IL-12 production of DCs induces the proliferation of $CD8^+$ CTLs and enhances IFN-γ, which can also increase MHC class I expression in many cell types and potentiate $CD8^+$ CTL-mediated killing. Taken together, these data demonstrate that yeast-CEA vaccination enhances the recruitment of immune-mediated cells to draining lymph nodes and increases the secretion of Th-1 and Th-2 cytokines and costimulatory molecules, which are necessary to induce a robust cell-mediated immune response against cancer cells (Bernstein *et al.*, 2008).

After demonstrating that yeast-CEA vaccination had a positive effect on murine DCs, our laboratory demonstrated

Table 1A. Effect of the yeast-CEA vaccine on the lymph node cell population, expression of costimulatory molecules, and production of cytokines

Murine DCs	Fold increase
A. Lymph node cell population	
$CD4^+$ T cells	3.4
$CD8^+$ T cells	2
$CD19^+$ B cells	2.4
APCs	3
B. Costimulatory molecules	
B7.1	1.4
LFA-3	1.2
C. Cytokines	
IL-12	2
TNF-α	84
IFN-γ	5
IL-10	4.5
IL-6	23

C57BL/6 mice ($n = 5$/group) were administered 1 YU of yeast-CEA/mouse subcutaneously in the right thigh on day 0 and sacrificed 2 days postvaccination. The total cells from draining inguinal lymph nodes were counted after lysis of red blood cells and stained for populations of B cells, $CD4^+$ T cells, $CD8^+$ T cells, and APCs (A). DCs derived from C57BL/6 mouse bone marrow were treated with GM-CSF and IL-4. On day 5, DCs were incubated with yeast-CEA for 48 h. Cells were analyzed by flow cytometry for the expression of costimulatory molecules (B) and supernatant fluids of harvested cells were analyzed by cytometric bead array for the expression of cytokines (C). Fold increase represents the difference between yeast-CEA treatment and control treatment groups (Wansley *et al.*, 2008).

Table 1B. Effect of *in vitro* treatment of human DCs with yeast-CEA on surface markers and cytokine production

Human DCs	Fold increase
A. Surface markers	
CD80 (B7.1)	4.1
CD54 (ICAM-1)	1.2
MHC class II molecules	1.2
B. Cytokines	
TNF-α	> 10 000
IFN-γ	> 6718
IL-8	> 162.4
IL-2	> 386.5
IL-13	12
IL-10	> 141

Human DCs were cultured for 48 h with yeast-CEA or media, and then harvested and analyzed by flow cytometry for surface-marker expression (A). Cultured supernatants were collected and screened for cytokine production (B) using the multiplex cytokine kit. Fold increase represents the difference between yeast-CEA treatment and control treatment groups (Remondo *et al.*, 2009).

for the first time that yeast-CEA could activate human DCs, resulting in increased surface expression of costimulatory molecules and MHC class II, and the production of inflammatory cytokines (Remondo *et al.*, 2009) (Table 1B).

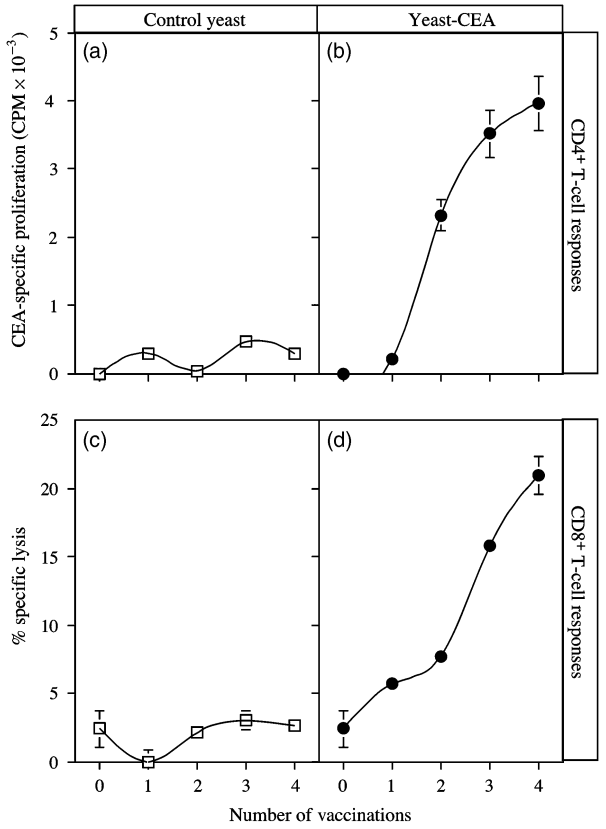

Fig. 3. Multiple vaccinations with yeast-CEA continuously increase the T-cell response. CEA-Tg mice were vaccinated with 0.1 YU of control yeast or yeast-CEA 1, 2, 3, or 4 times at 7-day intervals. Fourteen days after the last vaccination, mice were sacrificed, spleens were harvested, and splenocytes were used for assays. Open squares, control yeast; filled circles, yeast-CEA. (a) $CD4^+$ T-cell proliferation after vaccination with control yeast. Purified $CD4^+$ T cells were cultured with irradiated APCs and CEA protein for 5 days. [^{3}H]thymidine (1 μCi per well) was added to the wells for the last 24 h, and proliferation was assayed by measuring incorporated radioactivity. (b) $CD4^+$ T-cell proliferation after vaccination with yeast-CEA. (c) $CD8^+$ CTL activity after vaccination with control yeast. Splenocytes were stimulated with CEA peptide for 6 days before assays. Lymphocytes were incubated for 5 h with ^{51}Cr-labeled target EL-4 cells pulsed with CEA or VSV-NP control peptide. Radioactivity in the supernatant was measured and specific lysis was calculated. SD is based on the mean of triplicate wells. (d) $CD8^+$ CTL activity after vaccination with yeast-CEA. Data are presented as percent lysis after subtraction of the VSV-NP control (Wansley *et al.*, 2008).

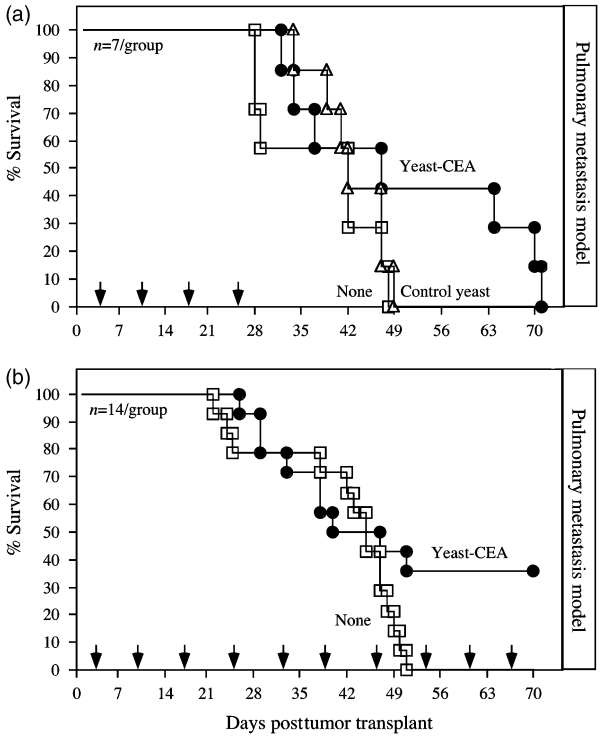

Fig. 4. Vaccination with yeast-CEA reduces tumor growth and increases the overall survival in tumor-bearing mice. (a) Survival in an experimental CEA^+ lung metastasis model. CEA-Tg mice ($n = 7$/group) were injected with 1×10^6 MC38-CEA^+ tumor cells intravenously in the tail on day 0 and mock-treated or injected with 1 YU control yeast or yeast-CEA subcutaneously on days 4, 11, 18, and 25 (arrows). Mice were monitored and survival was recorded. Open squares, no treatment; open triangles, control yeast; filled circles, yeast-CEA. (b) Survival in a lung metastasis model with continuous weekly vaccination (arrows). CEA-Tg mice ($n = 14$/group) were injected with 1×10^6 MC38-CEA^+ tumor cells intravenously in the tail on day 0 and mock-treated or injected with 1 YU yeast-CEA subcutaneously starting on day 4 and then weekly for the duration of the experiment. Mice were monitored and survival was recorded. Open squares, no treatment; filled circles, yeast-CEA (Wansley *et al.*, 2008).

Importantly, human DCs treated with yeast-CEA were able to generate CEA-specific T-cell lines capable of killing CEA^+ human tumor cells.

Several transgenic murine models expressing human CEA (CEA-Tg) have been developed to study the production of CEA-specific T-cell responses and antitumor activity against CEA^+ tumors. One CEA-Tg murine model was generated by microinjecting a 33-kb AatII restriction fragment containing the entire human CEA genomic region into a pronucleus C57BL/6 strain. Tissue-specific expression of the CEA protein was found predominantly in the gastrointestinal tract of the CEA-Tg mice, recapitulating the expression of CEA in humans, and making this animal an ideal model for studying the induction of CEA-specific immune responses in a self-antigen system (Clarke *et al.*, 1998). Using this CEA-Tg murine model, our laboratory conducted a more extensive preclinical evaluation of yeast-CEA vaccine (Wansley *et al.*, 2008), which led to five significant findings:

1. Recombinant yeast-CEA can break tolerance in this self-antigen animal model, as yeast-CEA vaccination in CEA-Tg mice generated CEA-specific $CD4^+$ and $CD8^+$ T-cell responses.

2. Yeast-CEA vaccination improved CEA-specific $CD8^+$ CTL responses in a dose-dependent manner (Fig. 2).

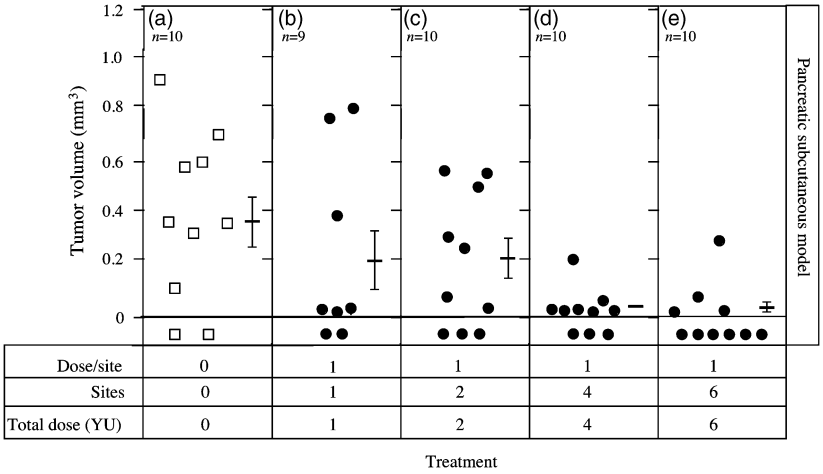

Fig. 5. Vaccination in multiple sites increases antitumor efficacy. CEA-Tg mice were implanted with 1×10^6 Panc02.CEA cells subcutaneously on day 0 and vaccinated in 0, 1, 2, 4, or 6 sites with 1 YU yeast-CEA/site starting on day 7 and then weekly for the duration of the experiment. Tumor volume was measured twice a week and recorded. (a) No treatment ($n = 10$). (b) 1 YU of yeast-CEA in one site ($n = 9$). (c) 1 YU of yeast-CEA in 2 sites ($n = 10$). (d) 1 YU of yeast-CEA in 4 sites ($n = 10$). (e) 1 YU of yeast-CEA in 6 sites ($n = 10$). Bars, average tumor volume ± SD; open squares, no treatment; filled circles, yeast-CEA (Wansley *et al.*, 2008).

3. Repeated administration of yeast-CEA enhances immune responses, as multiple administrations of yeast-CEA concomitantly increased CEA-specific $CD4^+$ and $CD8^+$ T-cell responses and significantly increased the overall survival of CEA^+ lung metastasis-bearing CEA-Tg mice (Figs 3 and 4).

4. Vaccination with yeast-CEA at multiple sites targeting different draining lymph nodes was more effective than single-site vaccination, as indicated by significant increases in $CD4^+$ T-cell responses and antitumor efficacy (Fig. 5).

5. CEA-Tg mice receiving the yeast-CEA vaccine mounted CEA-specific immune responses with no evidence of autoimmunity in this self-antigen system.

Taken together, these results indicate that yeast-CEA breaks tolerance in a self-antigen CEA-Tg mouse model and elicits robust therapeutic antitumor responses in the absence of autoimmunity. These findings led to the initiation of a phase I clinical trial evaluating the yeast-CEA vaccine in patients with CEA^+ tumors (NCI Clinical Trial 00924092; http://www.clinicaltrials.gov).

An open-label phase I trial of yeast-CEA in patients with metastatic CEA-expressing carcinomas is currently ongoing and carrying out recruitment, with approximately 28 patients expected to enroll (NCI Clinical Trial 00924092; http://www.clinicaltrials.gov). This trial will evaluate the safety of the yeast-CEA vaccine and its ability to generate CEA-specific $CD4^+$ and $CD8^+$ T-cell responses, as well as a clinical response, overall survival, and circulating tumor cells. As demonstrated in preclinical studies, multiple vaccination sites target multiple draining lymph nodes, thereby enhancing the activation of tumor-specific $CD4^+$ and $CD8^+$ T-cell responses needed to extend survival and/or reduce tumor burden. Therefore, patients will be given the yeast-CEA vaccine at four sites: the right and the left chest area below the armpit, and the right and the left upper thigh in the pelvic region. The vaccine will be administered in seven cycles of 14 days, on days 1, 15, 29, 43, 57, 71, and 85.

Yeast vaccine combined with chemotherapy

In recent years, the field of cancer immunotherapy has achieved several significant milestones due to the success of trials of the cancer vaccines sipuleucel-T and PROSTVAC-VF. Sipuleucel-T is an autologous DC-based vaccine. In a recent phase III clinical trial, patients with advanced prostate cancer ($n = 225$) randomized to receive sipuleucel-T demonstrated a 33% reduction in the risk of death and a significant increase in the median survival of 4.3 months compared with patients receiving placebo (23.2 vs. 18.9) (Higano *et al.*, 2009). PROSTVAC-VF is a viral-based vaccine composed of two recombinant viral vectors, each encoding transgenes for prostate-specific antigen, and three immune costimulatory molecules (B7.1, ICAM-1, and LFA-3). Recently published data from PROSTVAC-VF phase II trials in metastatic castration-resistant prostate cancer ($n = 125$) demonstrate that this vaccine is well tolerated and is associated with a 44% reduction in the death rate and an 8.5-month improvement in the median overall survival compared with placebo (Kantoff *et al.*, 2010).

Although both the sipuleucel-T and the PROSVAC-VF clinical trials have yielded significant clinical benefits, a mounting body of evidence suggests that cancer vaccines would probably be of the greatest benefit in the adjuvant or the neoadjuvant setting and/or where tumor burden is minimal (Schlom *et al.*, 2007; Gulley *et al.*, 2009). Large tumors have multiple, often redundant pathways to escape immune surveillance and mediate immune suppression, making them poor targets for immunotherapy. There is thus increasing interest in combining cancer vaccines with conventional standard-of-care (SOC) therapies, such as chemotherapy, that directly reduce tumor burden (Emens & Jaffee, 2005; Gulley *et al.*, 2009). Chemotherapeutic agents are known to be immunosuppressive; therefore, the traditional thinking has been that chemotherapy and

immunotherapy would not be an effective combination. Yet, while counterintuitive, recent evidence suggests that some chemotherapeutic agents can work synergistically to augment the antitumor effect of some immunotherapeutic agents, and thus generate superior antitumor activity than either modality alone (Zitvogel *et al.*, 2008; Gulley *et al.*, 2009; Higgins *et al.*, 2009). The induction of tumor-cell apoptosis by certain cytotoxic agents not only activates DCs but also provides them with an increased supply of tumor-specific antigens for presentation and cross-presentation to T cells. Additionally, several other immunostimulatory properties of cytotoxic drugs can work with immunotherapeutic agents to generate more robust immune-mediated cytotoxicity against malignant cells (Lake & Robinson, 2005). Furthermore, chemotherapy drugs are metabolized and eliminated, while the tumor-specific immunity induced by a therapeutic cancer vaccine is active, dynamic, and, more importantly, able to persist long after vaccination. Cancer vaccines thus have tremendous potential to confer protection against tumor recurrence. Altogether, the combination of chemotherapy and immunotherapy (particularly cancer vaccines) has many attractive benefits (Lake & Robinson, 2005; Higgins *et al.*, 2009). Ultimately, optimal dosage and scheduling of immunotherapy and chemotherapy are pivotal to the success of this multimodal therapy.

Yeast-ras and gemcitabine

Gemcitabine, a nucleoside analog, has been SOC for patients with advanced, inoperable pancreatic cancer for the last decade. Importantly, gemcitabine has been shown to modulate immune responses by reducing the frequency of myeloid suppressor cells and enhancing DC-dependent cross-presentation of tumor antigens to T cells (Nowak *et al.*, 2003a; Zitvogel *et al.*, 2008). The immunostimulatory benefits of gemcitabine have been demonstrated by enhanced tumor-specific CTLs and overall improvement in objective response rates in patients with pancreatic cancer, NSCLC, and colon cancer who received a combination of vaccines and recombinant IL-2 and granulocyte–macrophage colony-stimulating factor (Nowak *et al.*, 2003b; Levitt *et al.*, 2004; Plate *et al.*, 2005; Zitvogel *et al.*, 2008). Ongoing phase II clinical trials are evaluating the effect of combining yeast-ras and chemotherapy. A phase II double-blind, placebo-controlled, multicenter trial comparing yeast-ras vaccine plus six cycles of gemcitabine adjuvant vs. gemcitabine alone in patients with nonmetastatic, resected, ras-mutation$^+$ pancreatic cancer is ongoing and carrying out recruitment (NCI Clinical Trial 00300950, http://www.clinicaltrials.gov). An important enrollment criterion is that patients' tumor resection status must either be R0 (resection margin completely free of microscopic disease) or R1 (evidence of microscopic disease at the resection margin, but no macroscopic disease). This small tumor burden provides more time for both chemotherapy and immunotherapy to be efficacious. Prospective ras genotyping is performed to identify and match a patient's specific ras mutation with a yeast-ras vaccine. After tumor resection and before the initiation of gemcitabine therapy, patients receive three weekly doses of mutation-matched yeast-ras vaccine or placebo. Patients then receive six cycles of gemcitabine, with monthly injections of yeast-ras vaccine or placebo administered between gemcitabine cycles. The primary endpoint of this trial is recurrence-free survival, with overall survival as a key secondary endpoint (Britton *et al.*, 2009). This trial will determine whether gemcitabine and the yeast-ras vaccine can work synergistically to provide a meaningful clinical benefit to patients with ras$^+$ pancreatic cancers.

Yeast-CEA and cisplatin/vinorelbine

Cisplatin is a platinum-based chemotherapy drug that causes DNA cross-linking, interferes with mitosis, and results in apoptosis. It is used to treat various types of cancers, including NSCLC. Vinorelbine is another antimitotic chemotherapeutic drug used in NSCLC. Combinations of both drugs have been used as adjuvant chemotherapy following surgery in patients with NSCLC and have been shown to increase 5-year survival by 10–15% compared with no chemotherapy treatment (Gameiro *et al.*, 2008). A recent preclinical study in our laboratory demonstrated that appropriate scheduling of yeast-CEA and cisplatin/vinorelbine administration is crucial in this multimodal therapy (Gameiro *et al.*, 2008). The study showed that the yeast-CEA vaccine was most effective when not administered concurrently with cisplatin and vinorelbine, as both drugs can modulate the expression of immune cells. In particular, the study demonstrated a significant reduction in the population of CD4$^+$ and CD8$^+$ T cells, natural killer (NK) cells, and B cells 2 days after the administration of cisplatin/vinorelbine, compared with control-treated groups. However, after about 4 days, cell populations returned to baseline, indicating that this effect is transient and that these cells proliferated around 3 or 4 days after drug administration. The population of T-regulatory cells (Tregs), a subpopulation of T cells that suppresses the activation of the immune system and thereby maintains immune system homeostasis and tolerance to self-antigens, was also significantly reduced 2 days after the administration of cisplatin/vinorelbine. Interestingly, it appeared that the Treg population was more adversely affected by these drugs, because the cells did not return to baseline on day 4. This result demonstrated that appropriate scheduling is important for both the yeast-CEA vaccine and chemotherapeutic drugs. Clearly, the first yeast-CEA vaccination should be administered before chemotherapy is initiated, to prime and activate the immune

system. A second booster injection of yeast-CEA should be administered along with cisplatin/vinorelbine to take advantage of the approximately 3- to 4-day window when $CD4^+$ and $CD8^+$ T cells, NK cells, and B cells are proliferating and the Treg population is reduced. Using this scheduling strategy in an NSCLC mouse model demonstrated that the combination of cisplatin/vinorelbine and yeast-CEA vaccination was superior to either modality alone. Given this encouraging result, a phase II trial of yeast-CEA in combination with cisplatin/vinorelbine in patients with NSCLC is being planned. Patients with stages I–III NSCLC will undergo resection, and then receive three cycles of yeast-CEA during the 6-week rest period before adjuvant cisplatin/vinorelbine regimens. Patients will receive a yeast-CEA boost once a month during or between cisplatin/vinorelbine regimens for the duration of this trial. The primary endpoint is time to progression, with a secondary endpoint of overall survival and CEA-specific T-cell responses.

Yeast vaccine for infectious diseases

Yeast has also been used to produce proteins for vaccines to prevent infectious diseases caused by the hepatitis B and human papilloma viruses (McAleer *et al.*, 1984; Schiller *et al.*, 2008). For these vaccines, yeast is used to produce protein subunits or virus-like particles, respectively. One goal of prophylactic vaccines is to ensure presentation of the vaccine proteins to the immune system in such a way as to facilitate a T cell-dependent B cell-mediated antibody response able to produce neutralizing antibody titers directed against the virus of interest (Stanley, 2008). However, similar approaches to preventing hepatitis C virus (HCV) infections have been confounded by the immune evasion mechanisms used by HCV (Burke & Cox, 2010). Thus, therapeutic treatments for HCV infections are needed. The current SOC for HCV infections combines pegylated IFN-α with the small molecule antiviral ribavirin (Forde & Reddy, 2009). This therapy results in sustained virological responses in 50–75% of patients, but the adverse-event profile and lack of efficacy in some patients underscore the need for additional treatment strategies (Forde & Reddy, 2009).

A novel approach under clinical investigation for the therapeutic treatment of HCV-infected patients includes induction of HCV-specific T-cell immunity capable of reducing the HCV viral load. Interestingly, in some patients, HCV infection clears spontaneously without a medical intervention, presumably due to HCV-specific T-cell immune responses (Rehermann & Nascimbeni, 2005). Thus, strategies to induce HCV-specific T cells in HCV-infected patients could provide a meaningful therapeutic intervention for this hard-to-treat disease. One approach to inducing HCV-specific T-cell immunity uses yeast to express an NS3-core fusion protein of HCV (yeast-HCV). Vaccination with heat-killed yeast-HCV has led to the induction of HCV-specific T-cell responses in mice and humans (Haller *et al.*, 2005; Schiff *et al.*, 2007). Specifically, 71 HCV-infected patients who had partial responses or relapses with SOC therapies were enrolled in a placebo-controlled, dose-escalation, phase Ib trial of yeast-HCV administered subcutaneously (Schiff *et al.*, 2007). HCV-specific T-cell IFN-γ ELISPOT responses were observed in peripheral blood samples from 9/39 patients (23%) vaccinated with yeast-HCV, compared with 0/16 patients receiving placebo. Additionally, a nonsignificant reduction in the viral load (1 log) and normalization of alanine aminotranferase (ALT) levels (50%) were observed in yeast-HCV-vaccinated patients compared with patients in the placebo arm. Next, a randomized, open-label, phase II trial compared SOC with SOC plus yeast-HCV vaccination in 140 HCV patients (McHutchison *et al.*, 2009). The combination of SOC and yeast-HCV vaccination demonstrated a nonsignificant 15% reduction in the HCV viral load compared with SOC alone. Further, SOC with yeast-HCV resulted in a significant twofold improvement of ALT normalization in IFN-α-naïve patients compared with SOC alone. These results suggest that yeast-HCV can induce HCV-specific T-cell responses that may further reduce disease burden in HCV-infected patients.

Conclusion

While prophylactic vaccines provide protection against infectious diseases, therapeutic vaccines seek to eliminate established infected or malignant cells and prevent future recurrence. Yeast is a promising therapeutic vaccine vehicle due to its ability to generate robust cellular immune responses against malignant or virally infected cells. This review looked at two cancer vaccines that use yeast: yeast-ras and yeast-CEA. The yeast-ras vaccine demonstrated preclinical antitumor activity and induction of ras-specific T-cell responses in a majority of patients in a phase I trial (Franzusoff *et al.*, 2005; Munson *et al.*, 2008). A phase II trial of the combination of yeast-ras and gemcitabine is ongoing (Munson *et al.*, 2008). Similarly, preclinical data on yeast-CEA led to the initiation of a phase I clinical trial evaluating the yeast-CEA vaccine in patients with CEA^+ tumors. A phase II trial of yeast-CEA plus cisplatin/vinorelbine in patients with NSCLC is ongoing and recruiting patients (Gameiro *et al.*, 2008). Additionally, vaccination of HCV-infected patients with yeast-HCV led to the induction of HCV-specific T-cell responses (Schiff *et al.*, 2007; McHutchison *et al.*, 2009). Together, these data demonstrate that yeast can direct a therapeutic immune response to potentially improve outcomes for patients with cancer or chronic infections.

Future perspectives

In the future, therapeutic yeast vaccines may be used to target other cancers or infectious diseases, such as

melanoma and HIV (Barron *et al*., 2006; Riemann *et al*., 2007). As SOC treatments for cancers and infectious diseases change, there is a rationale for the strategic combination of treatments such as small molecule inhibitors, radiotherapy, antiangiogenesis, and hormone therapy with yeast vaccine to optimize clinical outcomes (Reits *et al*., 2006; Arlen *et al*., 2007; Chakraborty *et al*., 2008a, b; Hodge *et al*., 2008; Ferrara *et al*., 2009; Higgins *et al*., 2009; Kamrava *et al*., 2009). In addition, it has been shown that concurrent administration of yeast- and viral-based vaccines targeting the same antigen induces a more diverse T-cell population that leads to enhanced antitumor efficacy. This provides the rationale for future clinical studies investigating the concurrent administration of different vaccine platforms targeting a single antigen to enhance antigen-specific immune responses (Boehm *et al*., 2010). Finally, accumulating evidence suggests that using a cancer vaccine in the early stages of disease is much more effective at inducing antitumor immune responses and improving the overall survival compared with the use of vaccine in later-stage disease (Gulley *et al*., 2009). Given the favorable safety profile of yeast, treatment of cancer patients at earlier stages of disease would appear to be a reasonable approach.

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