Is the "3+3" Dose-Escalation Phase I Clinical Trial Design Suitable for Therapeutic Cancer Vaccine Development? A Recommendation for Alternative Design

Osama E. Rahma1,2, Emily Gammoh3, Richard M. Simon3, and Samir N. Khleif1,4

Abstract

Purpose: Phase I clinical trials are generally conducted to identify the maximum tolerated dose (MTD) or the biologically active dose (BAD) using a traditional dose-escalation design. This design may not be applied to cancer vaccines, given their unique mechanism of action. The FDA recently published “Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines.” However, many questions about the design of cancer vaccine studies remain unanswered.

Experimental Design: We analyzed the toxicity profile in 239 phase I therapeutic cancer vaccine trials. We addressed the ability of dose escalation to determine the MTD or the BAD in trials that used a dose-escalation design.

Results: The rate of grade 3/4 vaccine-related systemic toxicities was 1.25 adverse events per 100 patients and 2 per 1,000 vaccines. Only two of the 127 dose-escalation trials reported vaccine-related dose limiting toxicities, both of which used bacterial vector vaccines. Out of the 116 trials analyzed for the dose–immune response relationship, we found a statistically significant dose–immune response correlation only when the immune response was measured by antibodies ($P < 0.001$) or delayed type hypersensitivity ($P < 0.05$). However, the increase in cellular immune response did not appear further sustainable with the continued increase in dose.

Conclusions: Our analysis suggests that the risks of serious toxicities with therapeutic cancer vaccines are extremely low and that toxicities do not correlate with dose levels. Accordingly, the conventional dose-escalation design is not suitable for cancer vaccines with few exceptions. Here, we propose an alternative design for therapeutic cancer vaccine development.

Introduction

Traditionally, the first-in-human clinical trials conducted in oncology drug development are phase I safety-dose-seeking trials. Since the establishment of the Federal Food, Drug, and Cosmetic Act in 1938, all drug manufacturers are required to prove drug safety before marketing (1). A later amendment passed in 1962 required that not only safety must be demonstrated, but efficacy as well (2). These regulations created a strong motivation for the design of clinical trials. In the late 1970s, the guidelines for phase I trials of anticancer drugs were established, and they have remained largely unchanged to this day (3). Finding the maximum tolerated dose (MTD) was set as the primary goal for phase I trials, because at that time, most anticancer drugs were cytotoxic agents. These drugs exhibit a dose–toxicity relationship that was also considered to hold for efficacy. Therefore, the highest tolerated dose was assumed to be the most efficacious (4). Although toxicity is an important endpoint in phase I trials testing cytotoxic agents, newer drugs such as molecular targeted agents (MTA) are often characterized by a dose–response curve that plateaus at a dose less than the MTD (5–7). In such cases, the preferred effect can be provided without considerable toxicity. Furthermore, the antitumor effects of most successful recently developed targeted agents have been mediated via measurable pharmacodynamic effects on their targets. Therefore, identifying a biologically active dose (BAD) has become an important goal for phase I trials testing MTAs (6). Cancer vaccines have emerged as a promising novel modality and identifying their MTD and BAD using the traditional phase I design has been a challenge (8). Cancer vaccines produce their effect by modulating the immune system to target specific antigens on cancer cells. This mechanism of action is not expected to be dose related or to induce severe toxicity. Accordingly, the FDA realized the need to change the paradigm in early

1Vaccine Branch, National Cancer Institute, Bethesda, Maryland. 2Division of Hematology/Oncology, University of Virginia, Charlottesville, Virginia. 3Biometric Research Branch, National Cancer Institute, Rockville, Maryland. 4Georgia Health Sciences Cancer Center, Augusta, Georgia.

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Corresponding Author: Samir N. Khleif, Georgia Regents University Cancer Center, 1411 Laney Walker Blvd, Augusta, GA 30925. Phone: 706-721-6744; Fax: 706-721-0469; E-mail: skhleif@gru.edu

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cancer vaccine development (9). To date, there has been no extensive analysis of cancer vaccine toxicity or the dose-safety and dose–immune response relationships. In this paper, we have attempted to provide such analyses. We also describe an alternative design strategy for the conduct of early therapeutic cancer vaccine clinical trials, and we identify the circumstances in which a traditional dose-escalation design is appropriate.

Materials and Methods

Inclusion criteria and trials classification

To establish our database for analysis, we searched PubMed for all phase I, phase I/II, and pilot studies for therapeutic cancer vaccines conducted from 1990 through 2011, excluding all trials for combination treatments and studies that did not use the NCI Common Toxicity Criteria. The vaccine studies were classified according to vaccine type. Some studies used vaccines that fell under more than one category. To simplify the analysis, the method of delivery (e.g., dendritic cells) was used as the main guideline for classification.

Toxicity analysis

We reported the number of systemic vaccine-related grade 3/4 toxicities, number of treated patients, and number of administered vaccines by vaccine category. We then examined the dose–toxicity relationship in the dose-escalation trials, specifically looking for dose-limiting toxicities (DLT). Vaccine-related toxicities are defined as grade 3/4 toxicities that are possibly, probably, or definitely related to the vaccine, excluding local reactions, constitutional symptoms, and adjuvant-related events.

Translational Relevance

The traditional phase I dose-escalation design has been used by investigators in early therapeutic cancer vaccine development for decades. However, in contrast with cytotoxic agents, this design may not be applicable for therapeutic cancer vaccines given their unique mechanism of action and their relatively limited toxicities. The data presented in this report support the lack of correlation between dose escalation and cancer vaccines’ toxicities or cellular immune responses except for certain cases. In addition, this current report presents a novel design for therapeutic cancer vaccines’ early development. This design may allow investigators to conduct early-phase clinical trials in cancer vaccines without the need to enroll a large number of patients to identify the safe and immune active dose. Subsequently, the identified safe and immune active dose could be used in combination with other agents in phase II/III clinical trials. This would provide clinical investigators with a new tool to conduct clinical trials in cancer immunotherapy.

Dose–immune response meta-analysis

To address the question of whether dose escalation could lead to a better immune response, we performed a meta-analysis on all dose-escalation trials that reported the number of immune responders per dose level. Trials included in the meta-analysis were those dose-escalation trials analyzed earlier for toxicity in addition to phase II trials that used dose escalation to determine the dose that induces the best immune response as a primary endpoint.

Furthermore, we analyzed the correlation of immunologic response with dose level for each of the immune assay endpoints. This analysis included all studies that reported results with a given endpoint regardless of the type of vaccine. Statistical significance was based on a test for trend, which is essentially equivalent to an evaluation of whether the response (0 for no immunologic response vs. 1 for immunologic response) is linearly correlated with dose level (1, 2, etc.). For each study, a value $T_s = \sum_{i=1}^{n} y_i d_i$ was computed where $y_i$ denotes the response for the $i$th patient in the study, $d_i$ denotes the dose level (1, 2, etc.) of the $i$th patient in the study, and $n$ is the number of patients in the study. A large value of $T_s$ indicates a relationship of response to dose, but the size of $T_s$ depends on the number of patients in the study, the number of dose levels, and the distribution of patients per dose level. A standardized statistic was calculated for each study by subtracting off the mean and dividing by the square root of the variance under the null hypothesis of no trend of response to dose; that is, $T'_s = (T_s - E)/\sqrt{V}$. The studies were combined by computing an unweighted average of the $T'_s$ values, that is, $T'$. The statistical significance of $T'$ was evaluated by computing its exact permutation distribution. That is, for each study, the assignment of doses to patients was permuted randomly; new values of $T'_s$ and $T'$ were calculated for the permuted data. This was repeated thousands of times, resulting in the distribution of $T'$ under the null hypothesis. A one-tailed significance level is the area of the tail of the null distribution beyond the actual value of $T'$ for the real unpermuted data. A two-tailed significance level is taken as twice the one-sided value. The calculations were programmed in the R statistical programming language.

Results

Therapeutic cancer vaccine trials

We reviewed 239 phase I, phase I/II, and pilot therapeutic cancer vaccine studies published between 1990 and 2011. We classified these trials into cellular (autologous or allogeneic) and synthetic vaccines based on the type of vaccination. Cellular-based vaccines (autologous or allogeneic tumor cell-based vaccines) utilize the whole cells or cell lysates as the source of antigens, which allows multiple antigens to be simultaneously targeted without being prospectively identified. Autologous vaccines were subclassified into dendritic and tumor cell vaccines. Synthetic vaccines are administered directly to the patients or utilize a vector to deliver the antigen. Synthetic vaccines were
subclassified into peptide, DNA, RNA, viral, bacterial, anti-idiotypic, and liposomal vaccines. A full list of the 239 analyzed trials is reported in Supplementary Table S1, in addition to the range of the administered vaccine doses. An aggregate total of 4,952 patients were enrolled in these trials. Trials using synthetic vaccines accounted for the greatest number (135 trials), enrolling 2,853 patients, constituting more than half the total patients (57.61%). Autologous vaccines constituted 87 trials and 34.17% of the total patients (1,692 patients). There were 17 allogeneic vaccine trials with 407 treated patients (8.22% of the total patients; Table 1).

Vaccine-related toxicity

Vaccine-related toxicity in relation to the number of treated patients.

Here, we report the incidence of vaccine-related toxicity in relation to the number of treated patients (Table 1). We found that amongst the 4,952 patients assessed, a total of 162 grade three and 5 grade four treatment-related toxicities were reported. Of these toxicities, 60 were local reactions, 40 were constitutional symptoms, and 5 were related to the adjuvants used in the vaccines. The rest, 62 systemic adverse events, were reported by the investigators to be at least "possibly related" to the vaccine except for local reactions and constitutional symptoms.

Vaccine-related toxicity in relation to the number of administered vaccines.

We also evaluated the incidence of toxicities in relation to the total number of administered vaccines (Table 2). Only 206 of the 239 trials stated the total number of administered vaccines, and were included in this analysis. These 206 trials treated a total of 4,024 patients who received a total of 21,835 vaccines and experienced a total of 120 grade 3/4 toxicities. Of these 120 toxicities, 43 were systemic vaccine-related grade 3/4, accounting for two adverse events per 1,000 vaccines. Autologous vaccines had the lowest systemic vaccine-related toxicity rate (1.4 adverse events per 1,000 vaccines), followed by the synthetic vaccines (2.1 adverse events per 1,000 vaccines), and the allogeneic vaccines (2.6 adverse events per 1,000 vaccines).

Dose-related toxicity

To determine whether there is any relationship between dose and toxicity, we analyzed the dose–adverse event relationship in the trials that used a dose-escalation design (Table 3). Of the 239 trials, 127 used a vaccine dose-escalation design, treating a total of 2,985 patients. Amongst these 127 dose-escalation trials, only 22 (17%) reported toxicities of grade 3/4, a total of 98 events. Forty of these 98 events were systemic, non-constitutional, and vaccine related. To further investigate whether a dose–toxicity correlation existed, we performed two analyses:

### Table 1. Vaccine-related toxicities based on the number of treated patients

<table>
<thead>
<tr>
<th>Vaccine category</th>
<th>No. trials</th>
<th>No. patients</th>
<th>No. events</th>
<th>% Events</th>
<th>No. events</th>
<th>% Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous</td>
<td>87</td>
<td>1,692</td>
<td>37</td>
<td>2.19</td>
<td>23</td>
<td>1.36</td>
</tr>
<tr>
<td>DC</td>
<td>57</td>
<td>922</td>
<td>9</td>
<td>0.98</td>
<td>3</td>
<td>0.33</td>
</tr>
<tr>
<td>Tumor</td>
<td>30</td>
<td>770</td>
<td>28</td>
<td>3.64</td>
<td>20</td>
<td>2.60</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>17</td>
<td>407</td>
<td>22</td>
<td>5.41</td>
<td>5</td>
<td>1.23</td>
</tr>
<tr>
<td>Synthetic</td>
<td>135</td>
<td>2,853</td>
<td>108</td>
<td>3.79</td>
<td>35</td>
<td>1.23</td>
</tr>
<tr>
<td>Peptide</td>
<td>68</td>
<td>1,333</td>
<td>40</td>
<td>3.00</td>
<td>11</td>
<td>0.83</td>
</tr>
<tr>
<td>DNA</td>
<td>17</td>
<td>311</td>
<td>1</td>
<td>0.32</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td>RNA</td>
<td>2</td>
<td>36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Virus</td>
<td>30</td>
<td>662</td>
<td>23</td>
<td>3.47</td>
<td>13</td>
<td>1.96</td>
</tr>
<tr>
<td>Bacteria</td>
<td>6</td>
<td>126</td>
<td>27</td>
<td>21.43</td>
<td>7</td>
<td>3.97</td>
</tr>
<tr>
<td>Anti-idiotypic</td>
<td>10</td>
<td>362</td>
<td>15</td>
<td>4.14</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liposomal</td>
<td>2</td>
<td>23</td>
<td>2</td>
<td>8.70</td>
<td>2</td>
<td>8.70</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>4,952</td>
<td>167</td>
<td>3.37</td>
<td>62</td>
<td>1.25</td>
</tr>
</tbody>
</table>

**NOTE:** The number of grade 3/4 adverse events (AE) in relation to the number of treated patients.

aSystemic vaccine-related adverse events are all grade 3/4 toxicities possibly, probably, or definitely related to the vaccine except for local reactions and constitutional symptoms.
Vaccine-related toxicity in relation to the dose at which toxicities occurred. In the first analysis, we identified the dose level at which these 40 systemic vaccine-related toxicities occurred in each of the 22 trials. Seven of these 22 trials did not specify the dose at which the toxicities occurred (24 systemic toxicities; refs. 10–16). In the remaining 15 trials, only six reported the toxicities at the highest dose level (10 systemic toxicities). Two of these six trials that reported toxicities at the highest dose level used bacterial vaccines (17, 18), and the rest used autologous (19), DNA (20), viral (21), or liposomal vaccines (22). Two toxicities occurred in the middle dose level in one trial that used a bacterial vaccine, with no further toxicity occurring when the dose was escalated (23). Four toxicities occurred at the lowest dose level in three trials, two with peptide vaccines (24, 25) and one with a liposomal vaccine (22). Interestingly, none of these trials reported further toxicities when the dose was escalated (Supplementary Table S2).

Table 2. Vaccine-related toxicities based on the number of administered vaccines

<table>
<thead>
<tr>
<th>Vaccine category</th>
<th>No. trials</th>
<th>No. patients</th>
<th>No. vaccines</th>
<th>All grade 3/4 AE</th>
<th>Systemic vaccine-related grade 3/4 AEa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. events</td>
<td>% Events</td>
<td>No. events</td>
<td>% Events</td>
<td>No. events</td>
</tr>
<tr>
<td>Autologous</td>
<td>73</td>
<td>1,301</td>
<td>5,722</td>
<td>20</td>
<td>0.35</td>
</tr>
<tr>
<td>DC</td>
<td>51</td>
<td>796</td>
<td>3,424</td>
<td>9</td>
<td>0.26</td>
</tr>
<tr>
<td>Tumor</td>
<td>22</td>
<td>505</td>
<td>2,298</td>
<td>11</td>
<td>0.48</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>16</td>
<td>347</td>
<td>1,874</td>
<td>22</td>
<td>1.17</td>
</tr>
<tr>
<td>Synthetic</td>
<td>117</td>
<td>2,376</td>
<td>14,239</td>
<td>78</td>
<td>0.55</td>
</tr>
<tr>
<td>Peptide</td>
<td>61</td>
<td>1,183</td>
<td>7,637</td>
<td>37</td>
<td>0.48</td>
</tr>
<tr>
<td>DNA</td>
<td>15</td>
<td>259</td>
<td>1,388</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>RNA</td>
<td>2</td>
<td>36</td>
<td>335</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Virus</td>
<td>27</td>
<td>535</td>
<td>2,365</td>
<td>22</td>
<td>0.93</td>
</tr>
<tr>
<td>Bacteria</td>
<td>4</td>
<td>80</td>
<td>530</td>
<td>9</td>
<td>1.70</td>
</tr>
<tr>
<td>Anti-idiotypic</td>
<td>7</td>
<td>266</td>
<td>1,938</td>
<td>7</td>
<td>0.36</td>
</tr>
<tr>
<td>Liposomal</td>
<td>1</td>
<td>17</td>
<td>46</td>
<td>2</td>
<td>4.35</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>4,024</td>
<td>21,835</td>
<td>120</td>
<td>0.55</td>
</tr>
</tbody>
</table>

NOTE: The number of grade 3/4 adverse events (AE) in relation to the number of administered vaccines.
aSystemic vaccine-related adverse events are all grade 3/4 toxicities possibly, probably, or definitely related to the vaccine except for local reactions and constitutional symptoms.

Table 3. Dose–toxicity relationship

<table>
<thead>
<tr>
<th>Vaccine category</th>
<th>No. trials</th>
<th>No. patients</th>
<th>No. vaccines</th>
<th>Grade 3/4</th>
<th>No. systemic vaccine-related AEa</th>
<th>No. trials with DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. events</td>
<td>% Events</td>
<td>No. events</td>
<td>% Events</td>
<td>No. events</td>
<td></td>
</tr>
<tr>
<td>Autologous</td>
<td>40</td>
<td>847</td>
<td>2</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>DC</td>
<td>27</td>
<td>466</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tumor</td>
<td>13</td>
<td>381</td>
<td>2</td>
<td>11</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Allogeneic</td>
<td>5</td>
<td>130</td>
<td>3</td>
<td>20</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Synthetic</td>
<td>83</td>
<td>2,008</td>
<td>17</td>
<td>67</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Peptide</td>
<td>36</td>
<td>852</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>DNA</td>
<td>12</td>
<td>208</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Virus</td>
<td>26</td>
<td>592</td>
<td>7</td>
<td>47</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Bacteria</td>
<td>4</td>
<td>81</td>
<td>4</td>
<td>27</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Anti-idiotypic</td>
<td>8</td>
<td>339</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liposomal</td>
<td>1</td>
<td>17</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>2,985</td>
<td>22</td>
<td>98</td>
<td>40</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE: The number of grade 3/4 adverse events (AE) in dose-escalation trials and the number of trials that identified DLTs.
aSystemic vaccine-related adverse events are all grade 3/4 toxicities possibly, probably, or definitely related to the vaccine except for local reactions and constitutional symptoms.
**Trials with vaccine-related DLTs.** In the second analysis, we evaluated whether dose-escalation resulted in a DLT. Accordingly, we analyzed the 22 dose-escalation trials that reported grade 3/4 systemic vaccine-related toxicities for evidence of DLT. We found that only three of these 22 trials (15, 21, 26) reported DLTs (Tables 3 and 4). Amongst these, one trial, reported by Dols and colleagues, used allogeneic vaccine and attributed the DLT of nausea and vomiting to the adjuvant (GM-CSF). There were no life-threatening side effects on this trial (26). The two other trials, reported by Maciag and colleagues and Guthmann and colleagues, attributed the DLTs to the vaccines, both of which used bacterial vectors of live-attenuated Listeria monocytogenes and Neisseria meningitidis, respectively (15, 21). In both trials, the DLT was consists of hypotension that was successfully controlled with IV fluids and supporting medications. All the trials that used bacterial vector vaccines are described in Supplementary Table S3.

**Dose–immune response analysis**

To determine whether vaccine dose affects the resultant immune response, we reviewed the dose-escalation trials that reported the number of immune responders at each dose level. Only 106 of the 127 dose-escalation trials reported the number of immune responders at each dose level. We also included 10 additional phase II trials that used a dose-escalation design and tested for immune response as a primary endpoint. Accordingly, we identified 116 trials that could be analyzed for correlation between dose of the vaccine and immune response.

**Dose–immune response relationship based on the trials’ reports.** Out of these 116 trials, only two reported a statistically significant dose–immune response correlation. The immune response was measured by antibodies in both of these trials. One used an allogeneic vaccine (11) and the other used an anti-idiotypic vaccine (27).

**Dose–immune response relationship based on the meta-analysis results.** Furthermore, we analyzed the relationship of immunologic response with dose level based on the assay that was used to measure the immune response. These assays included delayed-type hypersensitivity (DTH), T-cell proliferation, IFN-γ ELISPOT, and tetramer assay in addition, at times, to humoral immune response. We found a statistically significant correlation between the vaccine dose and the immune response only when the immune response was measured by antibodies ($P < 0.001$) or DTH ($P < 0.05$). None of the other assays showed a trend that was statistically significant at a two-tailed 5% level. The results for tetramer assay and T-cell proliferation were of borderline significance, with a two-tailed significance level of about 10%. The borderline significance of the tetramer result was driven by a single study reported by Dangoor and colleagues that used plasmid DNA and a recombinant modified Vaccinia Virus Ankara (MVA), both expressing epitopes from five melanoma antigens (18). Without the Dangoor and colleagues study, the two-sided $P$ value for the remaining 10 studies did not approach significance. The borderline significance for T-cell proliferation was similarly non-robust. Only five studies were available that reported results for T-cell proliferation, all were small, and none showed strong evidence of correlation between the vaccine dose and the immune response. Removal of the study by Pinilla-Ibarz and colleagues (28), which used a bcr-abl fusion peptide vaccine, resulted in a two-tailed $P$ value of 0.26, which did not approach significance.

Because DTH was the only assay that showed a significant dos–cellular immune response correlation, we examined whether the percentage of DTH immune responders, on a specific trial, is increased with increasing the dose level (Fig. 1). A total of 19 trials reported the immune response by DTH. We found that the percentage of responders between dose level 1 and dose level 2 increased or maintained the same in 12 of these 19 trials, whereas it trended downward in 7 (Fig. 1A). Amongst the 12 trials that showed an increase or no change in the percentage of responders, eight trials continued to show an increase when the dose was escalated from dose level 2 to dose level 3 (Fig. 1B). However, only four of these eight trials continued to increase the dose to level 4 with only two of these four trials reporting an increased immune response by DTH (Fig. 1C). Although the number of patients per dose level in individual trials is limited, the data indicate the relationship between dose and DTH immune response, although statistically significant, was variable and inconsistent.

### Table 4. Trials with DLTs

<table>
<thead>
<tr>
<th>Trial</th>
<th>Vaccine used</th>
<th>Toxicity</th>
<th>DLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dols et al. (26)</td>
<td>Allogenic HER2/neu breast cancer cells (SC) with GM-CSF or BCG</td>
<td>Nausea vomiting</td>
<td>1 patient due to GM-CSF</td>
</tr>
<tr>
<td>Maciag et al. (15)</td>
<td><em>Listeria monocytogenes</em> secreting <em>HPV-16 E7</em> fused to <em>Lm listeriolysin O</em> (IV)</td>
<td>Hypotension</td>
<td>3 patients at highest dose level</td>
</tr>
<tr>
<td>Guthmann et al. (21)</td>
<td>GM3 ganglioside with <em>Neisseria meningitidis</em> outer membrane (IM)</td>
<td>Hypotension</td>
<td>1 patient at highest dose level</td>
</tr>
</tbody>
</table>

**NOTE:** Dose-escalation trials with DLTs.

Abbreviations: SC, subcutaneously; IV, intravenously; IM, intramuscularly.
Discussion

The traditional phase I dose-escalation clinical trial design has been implemented in the development of cancer vaccines for more than two decades. However, the utility of this design has been questioned by many investigators and regulatory agencies, including the FDA and the European Medicines Agency (9, 29). Although therapeutic cancer vaccines are generally expected to have a relatively safe profile and dose-independent efficacy, to date there has been no systemic evaluation that provides objective evidence to address this issue. Here, and for the first time, we provide evidence-based proof for limited serious adverse events and lack of dose-related toxicity for therapeutic cancer vaccines. We found the incidence of grade 3/4 vaccine-related systemic toxicities to be 1.25 per 100 treated patients and 2 per 1,000 administered vaccines. Moreover, vaccine-related DLTs were found in only two of the 127 dose-escalation toxicity-seeking clinical trials reviewed. Both of these clinical trials used bacterial vaccine vectors. Accordingly, we propose that, with the exception of bacterial vector vaccines, the traditional dose-escalation design to determine MTD is not warranted in the development of cancer vaccines. Noteworthy, all the cancer vaccine trials that we reviewed here were in early development and had no clinical efficacy. In addition, there is a possibility that there may be a correlation between dose escalation and local or constitutional symptoms because these were not taken into account in our dose–toxicity relationship analyses. However, these symptoms are not relevant to the determination of the DLT because they are all manageable toxicities.

Phase I trials may also be used to determine the BAD (6, 8). Our analysis of the included trials in this meta-analysis suggested that the vaccine dose does not increase the rate of the cellular immune response as measured by some immune assays. Although increasing the vaccine dose was associated with an increase in immune response rate, as measured by DTH in some trials, the increase in immune response did not appear consistent or sustainable with further dose escalation. Our analysis is limited by the fact that most of the trials used experimental immune assays that have not been validated to accurately evaluate the induced cellular immune response (30). It was also limited by the fact that these phase I trials were not designed with the objective of characterizing the relationship between dose and immunologic response or even determining whether there is a dose response. Furthermore, the relationship between dose escalation and the degree of the induced immune response was not addressed in this manuscript because only a minority of the reviewed trials addressed this point. Detecting a dose that is biologically active is different from detecting the minimal dose that is biologically active or determining whether there is a relationship between dose and immunologic response. We suggest that the standard $3 + 3$ phase I design is not generally adequate in detecting a minimal BAD or the relationship between dose and immunologic response in therapeutic cancer vaccines.

Need for alternative designs for early development of therapeutic cancer vaccines

The FDA has recently recognized the need for changing the way therapeutic cancer vaccine trials are being conducted (9). Our results, described above, support that conclusion. The incidence of DLT in such trials has been very low and consequently the use of designs developed for the detection of MTD is generally not appropriate.
A "Guidance for Industry: Clinical Considerations for Therapeutic Cancer Vaccines" has been recently issued by the FDA (9). That Guidance recognizes the inapplicability of the traditional 3+3 design but indicates that a dose-escalation design be used to determine the BAD and that the selection of the starting "safe" dose for cancer vaccines should be supported by preclinical and/or prior human safety data. Developing a safe starting dose based on preclinical testing is, however, not a feasible option in the majority of cancer vaccines due to the species-specific presentation and recognition of such antigens and variation in immune response activity. In addition, the results of our review suggest that the dose-escalation approach is problematic for most cancer vaccines because neither toxicity nor cellular immune response appears consistently related to dose.

Suggested alternative designs by others
Few alternative designs have been considered to replace the traditional dose-escalation 3+3 phase I design for cancer vaccines. Messer and colleagues described a combined phase I/II design where a phase I dose-escalation trial serves as an interim safety analysis before proceeding to phase II (31). On the other hand, Korn and colleagues described an initial accelerated phase design where one patient per dose level is treated until a biologic response occurs. After the first response is seen, cohorts of 3 to 6 patients are treated per dose level in a traditional dose-escalation phase (32). Hunsberger and colleagues described two stages of dose escalation to reach a molecular targeted endpoint. The first stage uses a 3+3 traditional escalation and the second is developed to continue to escalate the dose as long as the biologic response rate is increasing (33). Simon and colleagues described alternative phase II designs for early cancer vaccine trial development (34). The cancer vaccine clinical trial working group (CVCTWG) has proposed a proof-of-principle trial design that combines some aspects of phase I and II trials in patients "without rapidly progressive disease" (35). Importantly, all of these suggested alternative designs are using a dose-escalation method, which, as we have shown, rarely determines toxicity or biologic activity measured by cellular immune response.

New proposed alternative design

Assumptions in early cancer vaccines development. Here, we suggest an alternative design for cancer vaccine development. To determine the starting dose of the vaccine, we need to take into account the following assumptions: (i) A cellular immune response is necessary to induce clinical efficacy. Immune response had been used as a surrogate for cancer vaccine efficacy in clinical trials (36–39). Many investigators have shown that patients who generate a T-cell immune response are more likely to have longer survival compared with nonimmune responders (40–42). (ii) Cancer vaccine dose does not consistently correlate with either toxicity or with the prevalence of induction of cellular immune response. (iii) A vaccine as a single agent is not enough to induce clinical efficacy, and combination therapy is needed (43). Accordingly, it is crucial to optimize the cellular immune response generated by a cancer vaccine when designing an early-phase clinical trial by combining the vaccine with other agents such as immune modulators. On the basis of the above, our suggested design proposes to define a dose that can induce an immune response and then combine the selected dose with the appropriate immune modulating agents or other therapeutic modalities to obtain a better outcome.

Alternative clinical trial design for cancer vaccine

**Step 1. Determining a starting dose of a vaccine**
- Vaccine class that is used before and found to be toxic (e.g., bacterial vector) → Proceed to traditional phase I trial
- Vaccine class that is used before and found to be non-toxic (e.g., peptide) → Use IAD from previous clinical trials
- Vaccine class that is not used before and not expected to be toxic (e.g., peptide) → OPED
  - One patient per tested dose is treated until an immune response is induced (IAD).
  - Then, expand that dose level, one patient at a time, until achieving an additional immune response.
  - If no additional immune response in 7 patients, stop adding patients and continue escalation of one patient at a time.

**Step 2. Combination design “Vaccine + X”**
(X is an immune modulator, chemotherapy, or targeted agent)
- X had no DLT → Use the same dose
- X had a DLT → Use the dose below MTD
- X’ DLT is unknown → Proceed to traditional phase I

Figure 2. The suggested alternative design for early cancer vaccine development. IAD, Immune Active Dose; OPED, One Patient Escalation Design.
Step 1. Determining a starting dose of a vaccine. We propose a two-step design (Fig. 2). The first step is to determine an immune response–inducing dose. This would be used in step 2, a phase II trial testing the combination of cancer vaccine and immune modulating agents or other therapeutic modalities. Accordingly, the intention, first, is to determine a dose that can generate an immune response of a cancer vaccine, or Immune Active Dose (IAD). Because local adjuvant is often used in combination with the vaccine, both the vaccine and the adjuvant are considered one entity in our design. The method of determining the IAD will be dependent on whether the vaccine belongs to a class of vaccines that has been previously tested in humans. If the vaccine class has been administered in humans and found to be nontoxic (e.g., a peptide), then we propose using the same IAD shown in previous trials in a phase II combination approach with immune modulators and/or other therapies. On the other hand, if the vaccine belongs to a class that had been used before and found to be toxic in humans (e.g, bacterial vectors, as shown above), or a new self-antigen that is crucial for a vital organ or function (e.g., angiogenesis antigens), then we recommend using the traditional 3+3 dose-escalation design.

Finally, if the tested vaccine is considered "novel" or belongs to a class that has not been tested before and is expected to be nontoxic, we recommend using a "One Patient Escalation Design (OPED)" to determine the dose of the antigen that is active in at least one patient. By using the OPED, one patient per tested dose is treated until an immune response is induced to determine the IAD. To confirm the dose as the IAD, the same dose will be administered to an expanded group of patients. Having a precise estimate of the immunologic response rate at that dose is not the goal; rather the goal is to confirm that there is some biologic activity at that dose. We propose the following approach: if the expected cellular immune response rate, with the vaccine alone at that dose is 30%, the probability of obtaining no responses in an expansion cohort of 7 patients is 0.082. Therefore, from this perspective, to make sure that the initial response was real, it would be appropriate to expand the cohort at that dose level, one patient at a time, until achieving an additional response. If no additional response is seen in 7 patients, then we will stop adding patients and will continue escalation of one patient at a time until we obtain an immunologic response with a confirmatory expansion cohort. However, if an additional immune response is observed in the expanded cohort, no more patients will be enrolled because the purpose of adding more patients is to confirm the first immune response.

If the probability of immune response is denoted by p and is independent of dose, then the number of patients treated until the first response is observed has a geometric distribution; the probability that the first response occurs at the nth patient is \( pq^{n-1} \) where \( q = (1 - p) \). The probability that the IAD will be confirmed by studying up to 7 patients at the dose level giving a response is \( 1 - q^7 \). When \( P = 0.33 \), the probability that the IAD occurs at the first dose level is 0.31 and the mean dose level at which it occurs is 3. The probability that the IAD is confirmed is 0.94. When \( P = 0.5 \), the probability that the IAD occurs at the first dose level is 0.496 and the mean dose level at which it occurs is 2. The probability that the IAD is confirmed is 0.99. If there is a dose–immunologic response relation, then the probability that the first response occurs at the nth patient is \( q(n-1)p_n \).

Step 2. Combining the vaccine with immune modulating agents or other therapeutic agents. The second step is to combine the vaccine with immune modulating agent, chemotherapeutic agent, or other cancer therapy agent referred to as "X" to enhance the cancer vaccine efficacy. During this step, the vaccine and the adjuvant are considered one entity and referred to as "vaccine." During this step, we recommend using the vaccine's IAD that is identified in the first step and setting the "X" dose based on its safety profile. If "X" had no known DLT, then we recommend combining the vaccine with the same "X" dose that was used before. On the other hand, if "X" has a known DLT then we recommend combining the vaccine with an "X" dose that is below its MTD. However, if the "X" DLT is unknown (testing a novel agent), then we recommend proceeding to a traditional phase I (3+3) design by escalating the "X" dose in combination with a fixed dose of the vaccine (IAD). Enhancing the cancer vaccine efficacy further may require adding another agent "Y" (e.g., immune modulator, chemotherapeutics, or targeted agent) to the combination of (vaccine + X). In this case, the combination of vaccine and X should be treated as one entity (vaccine + X) and the "Y" dose could be changed on the basis of its safety profile in a similar method that is used when combining the vaccine with "X." More agents could be added to the combination of vaccine + X + Y in a similar fashion (Fig. 2). Simon and colleagues have described a variety of phase II designs that can be used for optimizing a vaccine-based regimen (34).

Advantages and limitations of the new proposed design. Our suggested design has many advantages, including the ability to identify the safe and IAD without the need to enroll a large number of patients unless the study is testing a vaccine class that is known to be toxic. Our design also allows for enhancing the vaccine efficacy by combining the vaccine with other agents. However, our recommendation may have some limitations because it does not take into account the possible cumulative toxicities of cancer vaccines and the lack of validation of immunologic assays. Nevertheless, our suggested design may represent a step forward in therapeutic cancer vaccine development and needs to be tested in the future.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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