Chemotherapy Can Enhance the Therapeutic Potential of Vaccine-Mediated Immunotherapy

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NCI Center for Cancer Research
Reducing the Burden of Cancer Through Exploration, Discovery and Translation

Abstract

Purpose: Many non-small cell lung cancer (NSCLC) patients undergo surgery followed by standard-of-care adjuvant chemotherapy, which includes cisplatin in combination with vinorelbine. In spite of therapy, the median survival of patients with metastatic disease is less than 10 months. Previous studies have shown that chemotherapy given prior to vaccine can inhibit vaccine-mediated antitumor immunity. Since chemotherapy is standard of care for many cancer types, the possibility that chemotherapy can be used concomitantly with vaccine was evaluated. Certain chemotherapy regimens induce transient pancytopenia, which is followed by a recovery phase. We hypothesized that administering vaccine during the T-cell recovery phase would enhance the effectiveness of the vaccine. ***Experimental Design:*** We examined the effect of chemotherapy on the growth, Fas cell-surface expression, and CTL-mediated cytolysis of mouse Lewis lung carcinoma cell lines in vitro. We evaluated the potential for biological synergism between the standard-of-care chemotherapy regimen and a recombinant yeast-CEA vaccine in a mouse model of NSCLC. Moreover, we examined the effects of chemotherapy on the quantity and function of regulatory splenic T-cells.

Results: These studies demonstrate for the first time that (*a*) the combination of cisplatin plus vinorelbine modulates $CD4^+$, $CD8^+$, $CD19^+$, natural killer, and regulatory T-cell populations in healthy mice; and (*b*) cisplatin plus vinorelbine combined with heat-killed recombinant yeast-CEA vaccine (*t*) is superior to either modality alone at reducing tumor burden and (*ii*) increases vaccine mediated antigen-specific CD4 and CD8 T-cell responses. Moreover, cisplatin plus vinorelbine modulates the cell surface expression of immunologically relevant molecules and improves antigen-specific CTL mediate cytotoxicity in vitro. ***Conclusions:*** These finding suggest potential clinical benefit for the combined use of recombinant yeast vaccine and cisplatin-based chemotherapy regimens.

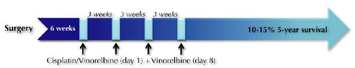
Rationale

Non-Small Cell Lung Cancer (NSCLC)

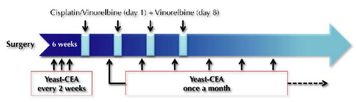
- ✔ Accounts for over 85% of all new lung cancer cases
- ✔ Half of all new cases present locally advanced or metastatic disease

Treatment

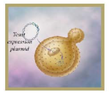
- ✔ Stage-dependent
- ✔ Patient Population
  - Stage IB-III resectable NSCLC
  - Immunocompetent
  - p.s. 0-1
  - Surgery plus adjuvant chemotherapy (platin-based) - *e.g.* cisplatin + vinorelbine



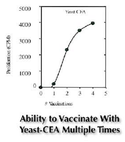
Can we combine chemotherapy with yeast-CEA vaccine?

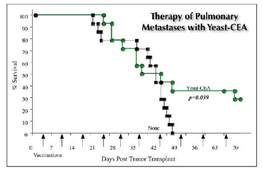


Recombinant *Saccharomyces cerevisiae*–CEA (Yeast-CEA) Vaccine

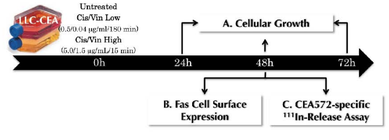


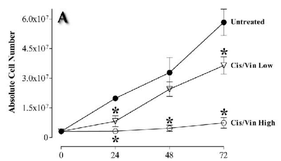
- *Cultured rapidly in large quantities*
- *Heat-killed, safe, and with no biosafety requirements*
- *Excellent safety profile in clinical studies [ras, and hepatitis C trials (n=80): no SAEs, no DLTs]*
- *Can be administered multiple times*

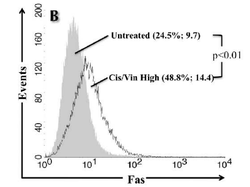




Results







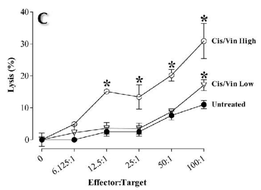


Figure 1. Effect of chemotherapy on the (A) growth, (B) cell-surface expression of Fas, and (C) sensitivity to CTL-mediated killing. LLC-CEA$^+$ cells were exposed in vitro to a high dose (*open circles*) of cisplatin (5 μg/ml) and vinorelbine (1.5 μg/ml) for 15 min. Alternatively, cells were exposed for 3 hours to a low dose (*open triangles*) of cisplatin (0.5 μg/ml) and vinorelbine (0.04 ug/ml), or left untreated (*closed circles*). ***A.*** At various time points after exposure, the total number of viable LLC-CEA$^+$ cells was determined by trypan blue exclusion. Results are representative of two independent experiments and are presented as mean±SD from 3 replicate flasks. ***B.*** Forty-eight hours after exposure, cells were harvested, stained for Fas, and analyzed by flow cytometry. Values indicate percentage of positive cells. Results are representative of two independent experiments. ***C.*** Forty-eight hours after exposure, cells were harvested and labeled with ^{111}In. The sensitivity of LLC-CEA$^+$ target cells to CEA572-specific-mediated killing was determined. After cells were incubated at different effector:target ratios for 18h, supernatants were harvested and specific lysis was calculated. Results are presented as mean±SD from 3 replicate wells. Stars denote statistical significance ($p<0.05$) relative to untreated cells.

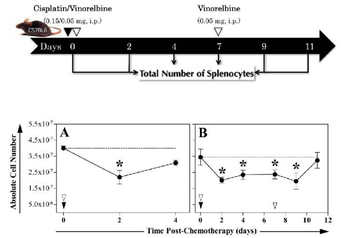
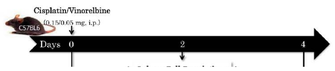


Figure 2. Effect of chemotherapy on the absolute number of splenocytes. At various time points after the administration of **(A)** a single dose of cisplatin/vinorelbine (▼▽)(0.15mg/0.05 mg, i.p.) on day zero, or **(B)** combined with a subsequent dose of vinorelbine (▽)(0.05 mg, i.p.) on day 7, the total number of splenocytes were determined. Results are representative of two independent experiments. Results are presented as mean±SD from 2-5 mice. Solid lines represent treated animals. Doted lines represent the mean number of splenocytes in untreated animals. Stars denote statistical significance ($p<0.05$) relative to untreated animals, as determined by 1-ANOVA with Bonferroni multiple comparison post-hoc test.



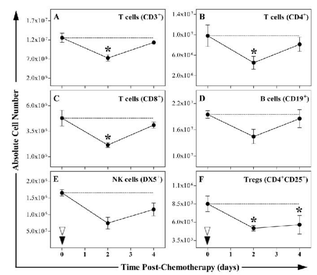


Figure 3. Effect of chemotherapy on the absolute number of spleen cell populations. At various time points after the administration of a single dose of cisplatin/vinorelbine (▼▽)(0.15mg/0.05 mg, i.p.) on day zero the total number of indicated populations were determined by flow cytometry. Results are representative of two independent experiments. Results are presented as mean±SD from 2-5 mice. Solid lines represent treated animals. Doted lines represent the mean number of cells in untreated animals. Stars denote statistical significance ($p<0.05$) relative to untreated animals.

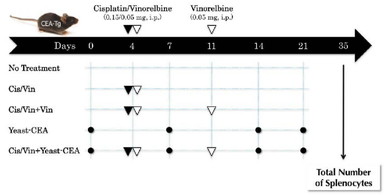


Table 1. Combination Therapy with Vaccine and Chemotherapy Results in Increased Antigen Specific T-cell Responses

Vaccine	Chemotherapy	$CD4^+$ T-cell Responses Myo	$CD4^+$ T-cell Responses CEA	Tetramer$^+$-$CD8^+$ T-cells per $1x10^6$ $CD8^+$ T-cells HIV	Tetramer$^+$-$CD8^+$ T-cells per $1x10^6$ $CD8^+$ T-cells CEA
None	None	187	1440	770	7076
None	Cisplatin/Vinorelbine Day 4	195	1375	705	6933
None	Cisplatin/Vinorelbine Day 4 Vinorelbine Day 11	151	471	229	7162
Yeast-CEA Day 0, 7, 14, 21	None	219	707	448	**7691**
Yeast-CEA Day 0, 7, 14, 21	Cisplatin/Vinorelbine Day 4	436	**4350**	654	**8091**

CEA-Tg mice (n=5/group) were not treated, or treated with Yeast-CEA on days 0, 7, 14, and 21 (1 YU/site, 4 sites) as indicated. Subgroups of mice recieved chemotherapy with cisplatin and vinorelbine on day 4 or cisplatin and vinorelbine on day 4 and vinorelbine on day 11. On day 35, mice were sacrificed, spleens were harvested, and analyzed for CEA-specific $CD4^+$ T-cell proliferation or CEA-tetramer$^+$ $CD8^+$ T-cell populations by flow cytometry.

For proliferation: antigen concentrations were: Con A (2.5 μg/ml); human myoglobulin (50 μg/ml) or CEA (50 μg/ml). All Con A values were >2.3x10^5 CPM. Each value represents the mean CPM of triplicate samples.
Bold numbers: significant increase versus no treatment, no chemotherapy (<0.01) using unpaired student T-test. Significant differences in flow cytometry data were determined using the Kolmogorov-Smirnov test.

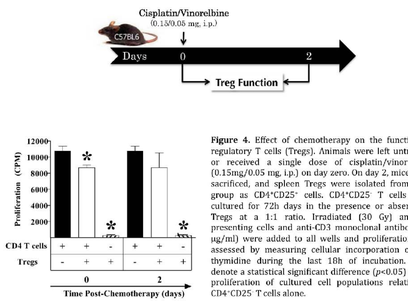
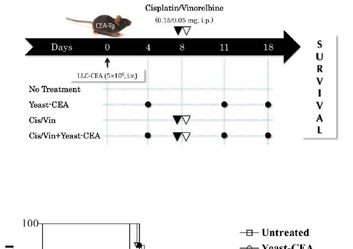


Figure 4. Effect of chemotherapy on the function of regulatory T cells (Tregs). Animals were left untreated or received a single dose of cisplatin/vinorelbine (0.15mg/0.05 mg, i.p.) on day zero. On day 2, mice were sacrificed, and spleen Tregs were isolated from each group as $CD4^+CD25^+$ cells. $CD4^+CD25^-$ T cells were cultured for 72h days in the presence or absence of Tregs at a 1:1 ratio. Irradiated (30 Gy) antigen-presenting cells and anti-CD3 monoclonal antibody (1 μg/ml) were added to all wells and proliferation was assessed by measuring cellular incorporation of 3H-thymidine during the last 18h of incubation. Stars denote a statistical significant difference ($p<0.05$) in the proliferation of cultured cell populations relative to $CD4^+CD25^-$ T cells alone.



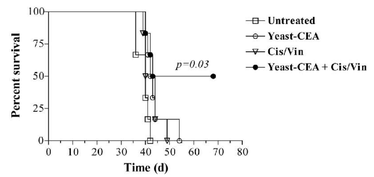


Figure 5. Combination of cisplatin/vinorelbine chemotherapy with vaccine increased the survival of tumor-bearing mice. CEA-Tg mice were inoculated i.v. with 5×10^5 LLC-CEA$^+$ cells on day 0. Control animals (n=6) were left untreated (*open squares*). A second group (*open circles*) was vaccinated on day 4 and boosted every 7 days thereafter with 4 YU of yeast-CEA vaccine (1YU/site, 4 sites). A third group (*open triangles*) received a single dose of cisplatin/vinorelbine (0.15/0.05 mg, i.p.) on day 8. The fourth group (*closed circles*) was vaccinated on day 4 and every week thereafter with 4 YU of yeast-CEA vaccine (1YU/site, 4 sites); on day 8, animals received a single dose of cisplatin/vinorelbine (0.15/0.05 mg, i.p.). All animals were monitored daily for survival during 68 days. Data is presented as percent survival. Statistical differences between treated and control groups were assessed using the Log-rank Test.

Future Plans

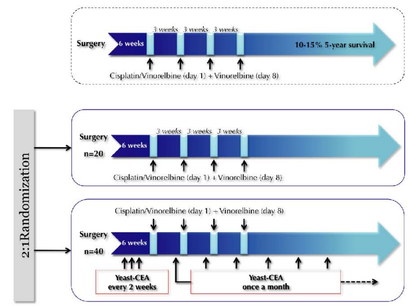
Phase II Trial

Saccharomyces-CEA Vaccine in Patients with Stage IB-III NSCLC Following Surgery

Patient Population: Stage IB-III resectable NSCLC, immunocompetent, p.s. 0-1; adjuvant chemotherapy (platin-based)

Primary Endpoint: Time to progression

Secondary Endpoint: Overall survival; CEA-specific CD8 T cells (ELISPOT), Tregs



Drs. R. Madan, P. Arlen, J. Gulley, P. Dennis, G. Glaccone