

A Randomized Phase I/II Trial Using a
GM-CSF-Producing and CD40L-
Expressing Bystander Cell Line
(GM.CD40L) Vaccine in Combination with
CCL21 for Patients with Stage IV
Adenocarcinoma of the Lung

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OVERVIEW

- Background - NSCLC
- Environment at MCC
- Background – Prior vaccine trials with GMCD40L and trials with CCL21
- Rationale
- Study Design and Objectives

Estimated New Cases*

			Males	Females			
Prostate	234,460	33%			Breast	212,920	31%
Lung and Bronchus	92,700	13%			Lung and Bronchus	81,770	12%
Colon and Rectum	72,800	10%			Colon and Rectum	75,810	11%
Urinary Bladder	44,690	6%			Uterine Corpus	41,200	6%
Melanoma of the Skin	34,260	5%			Non-Hodgkin Lymphoma	28,190	4%
Non-Hodgkin Lymphoma	30,680	4%			Melanoma of the Skin	27,930	4%
Kidney and Renal Pelvis	24,650	3%			Thyroid	22,590	3%
Oral Cavity and Pharynx	20,180	3%			Ovary	20,180	3%
Leukemia	20,000	3%			Urinary Bladder	16,730	2%
Pancreas	17,150	2%			Pancreas	16,580	2%
All Sites	720,280	100%	All Sites	679,510	100%		

Estimated Deaths

			Males	Females			
Lung and Bronchus	90,330	31%			Lung and Bronchus	72,130	26%
Colon and Rectum	27,870	10%			Breast	40,970	15%
Prostate	27,350	9%			Colon and Rectum	27,300	10%
Pancreas	16,090	6%			Pancreas	16,210	6%
Leukemia	12,470	4%			Ovary	15,310	6%
Liver and Intrahepatic Bile Duct	10,840	4%			Leukemia	9,810	4%
Esophagus	10,730	4%			Non-Hodgkin Lymphoma	8,840	3%
Non-Hodgkin Lymphoma	10,000	3%			Uterine Corpus	7,350	3%
Urinary Bladder	8,990	3%			Multiple Myeloma	5,630	2%
Kidney and Renal Pelvis	8,130	3%			Brain and Other Nervous System	5,560	2%
All Sites	291,270	100%			All Sites	273,560	100%

Introduction

- Lung cancer is the second most common cancer in men and women.
- Yet, it is the leading cause of cancer deaths.
- Most patient present with late Stage (III or IV) disease and are not candidates for curative therapy.
- Of those with early stage (Stage I-III A) disease, the majority will succumb to recurrent disease.

Stage	N	5 Yr survival (%)
IA	687	50
IB	1189	43
IIA	29	36
IIB	357	25
IIIA	511	14
IIIB	1030	10
IV	1427	6

TREATMENT

- Second-line therapy yields response rates of ~10%.
- More advancements are needed in this area
- Novel treatment modalities, including immunotherapy, need to be developed given the lack of significant advances using conventional approaches.

Background

- Tumors evade the immune system despite the presence of tumor associated antigens.
- Urgency to develop and augment tumor vaccines for increased anti-tumor immune responses.
- 2004 – GMCD40L Bystander Tumor Vaccine- Created at MCC
 - Secrete GM-CSF - recruit professional APCs in the form of DCs
 - Express CD40 Ligand- activate DCs
- 2006 – Phase-I trial on Stage IV cancer patients
 - GM.CD40L vaccine was found to be a safe method to deliver anti-tumor cell immune responses and was found to diminish disease burden in a variety of solid tumors

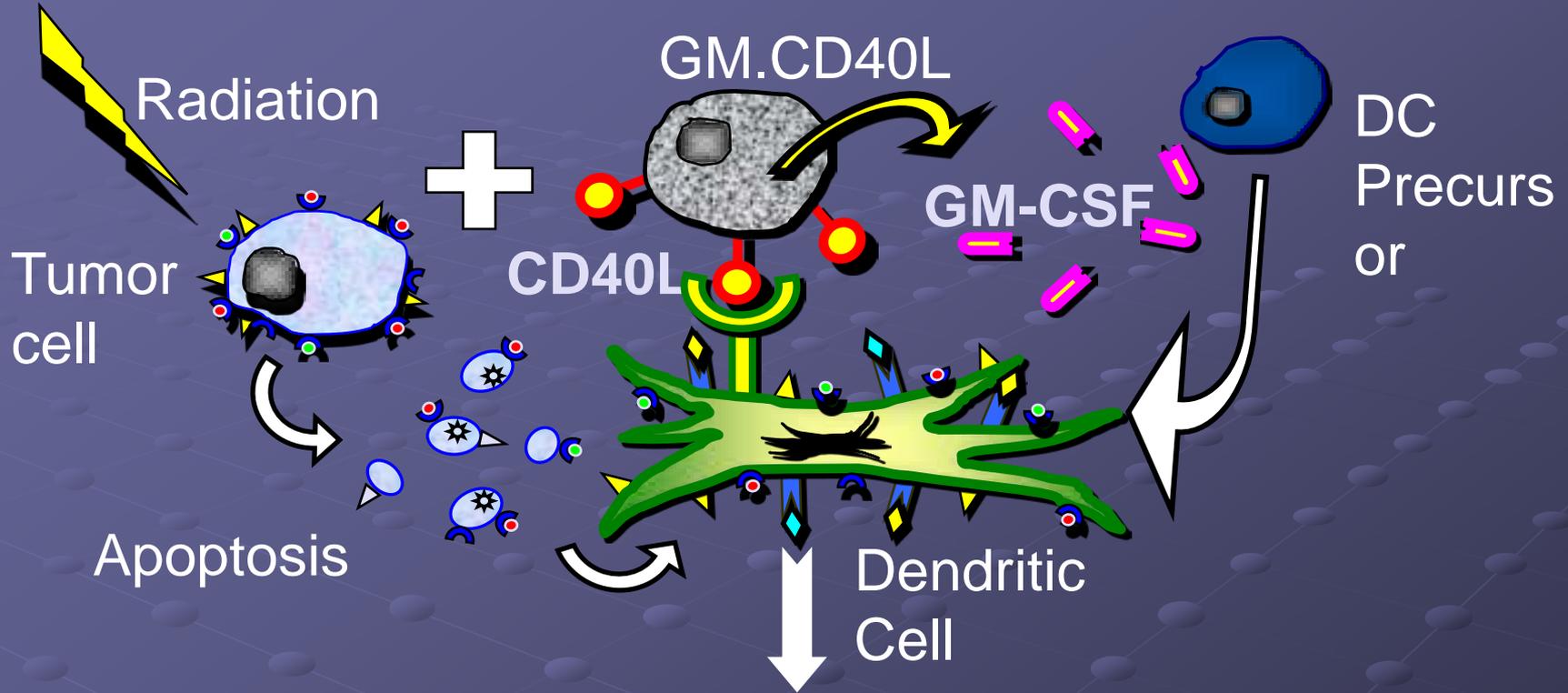
ENVIRONMENT

- Moffitt Cancer Center and Research Institute - MCC
 - Free Standing, NCI designated Cancer Center
- Immunotherapy Program/Thoracic Oncology Program
 - Dr. Scott Antonia
- Cell Therapy Core Facility
 - Dr. William Janssen
 - Practice under Good Manufacturing Practices-compliant conditions
 - Supervise Good Laboratory Practices-compliant conditions

Cell Bank Qualifications and Identification Testing

- Master Cell Banks
 - Manufacturer's working Cell Banks
 - Lots to lot generations
- Sterility/Adventitious agents
 - Bacteria, viruses, fungi, mycoplasma
 - Stability
 - Irradiation/freeze/thaw
 - Identification
 - Negative MHC (FC)
 - + CD40L expression (FC)
 - +GMCSF secretion (ELISA)

K.GM.CD40L Model



Mature, activated dendritic cells migrate to regional lymph nodes and activate resting T cells. These T cells can then circulate and kill cancer cells throughout the body.

Phase I Study: GMCD40L in solid tumors

- GM-CSF-producing and CD40L-expressing bystander cell line (GM.CD40L) and autologous tumor cells.
- 26 patients -enrolled
- 21 -patients treated
- No adverse effects (other than mild local inflammation at the site of vaccine injection) have been found.
- 4 patients – early progression
- 1 patient withdrew consent
- Immunoassays and other correlative studies demonstrated tumor specific T-cell responses, and recruitment and activation of dendritic cells, with evidence of stable disease in some patients including one lasting up to 24 months

A Phase II study of an allogeneic GM.CD40L vaccine in combination ATRA and cyclophosphamide in advanced NSCLC

- ATRA helps with DC maturation
- Cyclophosphamide's role was to inhibit T-regulator cells.
- 24 participants – enrolled
- Stopped early as it did not meet its primary endpoint of response rate.
- Only a few grade 3 events occurred
- 3 subjects: headaches
- 1 patient with diarrhea, nausea, and vomiting.
- In this heavily pretreated population of patients with NSCLC, we now have 2/24 patients enrolled who are long term survivors (30 months plus).

Outcomes and Comparisons

- Upon further review of the data, the median overall survival of the patients was 9 months despite this being a heavily pretreated population.

Outcomes and Comparisons

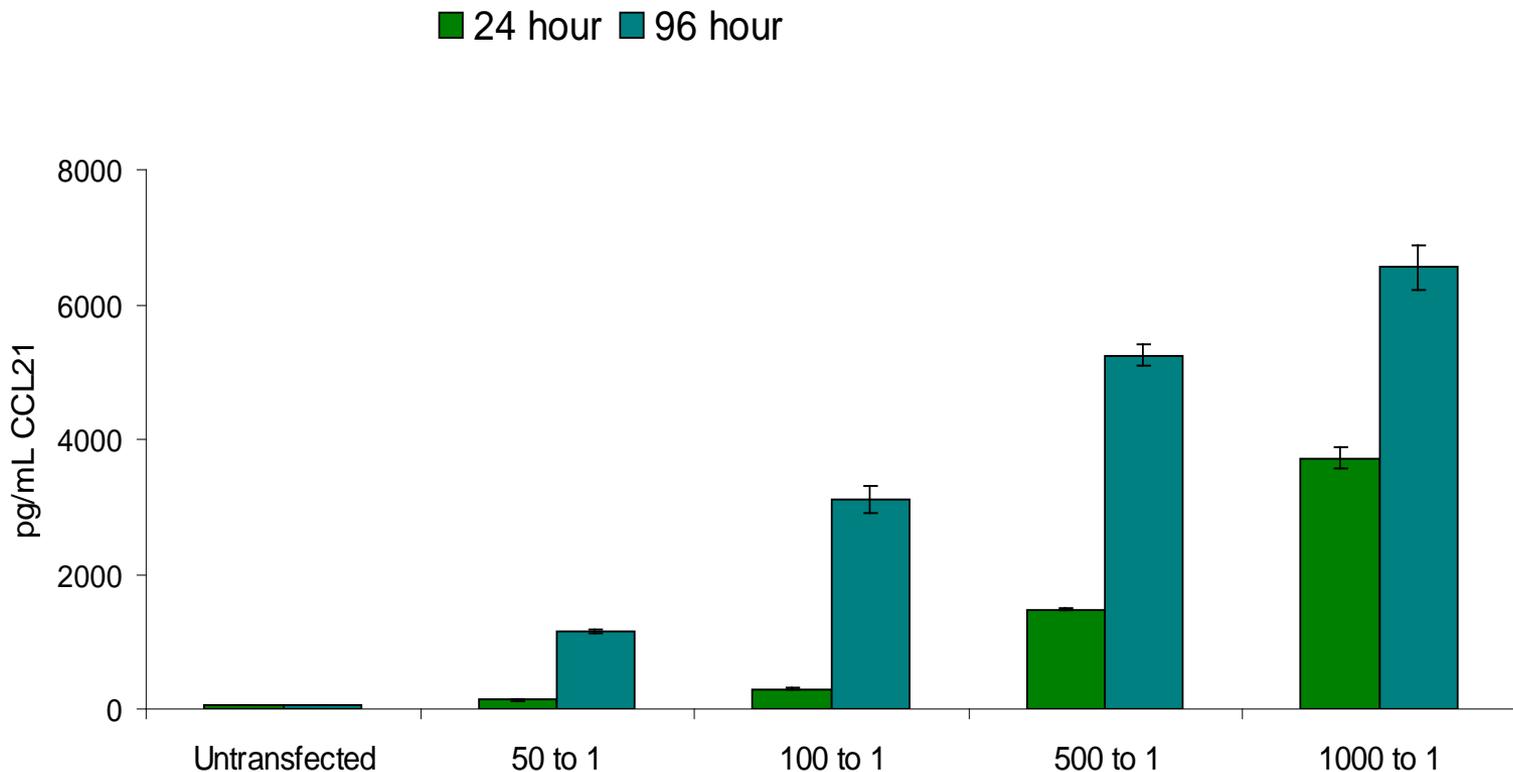
- Upon further review of the data, the median overall survival of the patients was 9 months despite this being a heavily pretreated population.
- When compared with historical studies
- Hanna et al study presented in JCO in 2004 our numbers are comparable if not better.
- On an intent-to-treat basis, the median survival time for pemetrexed was 8.3 months versus 7.9 for docetaxel (HR, 0.99; 95% CI, 0.82 to 1.2; noninferiority P : 0.226) which is similar to our median survival time of 9 months.
- Furthermore, with only one patient out of nearly 571 patients treated in both arms alive at 20 months while in our phase II study we have 2 out of 24 patients alive at 30+ months.

CCL21: (Exodus-2, SLC, 6Ckine)

- CC chemokine found in the high endothelial lymph nodes and T cell areas of spleens.
 - CCR7
 - CXCR3
- CD4⁺ CD25⁺ Regulatory T cells are hyporesponsive to CCL21. Flannagan, K et al. (2004) *Cellular Immunology*
- Mice lacking CCL21 expression have defects in lymphocyte homing and dendritic cell localization. Gunn, M.D. et al. (1999) *J. Exp. Med*
- Dendritic cell vaccine secreting CCL21 shows promise for chemotherapy resistant melanoma patients. Mule (2009) *Ann N Y Acad Sci*

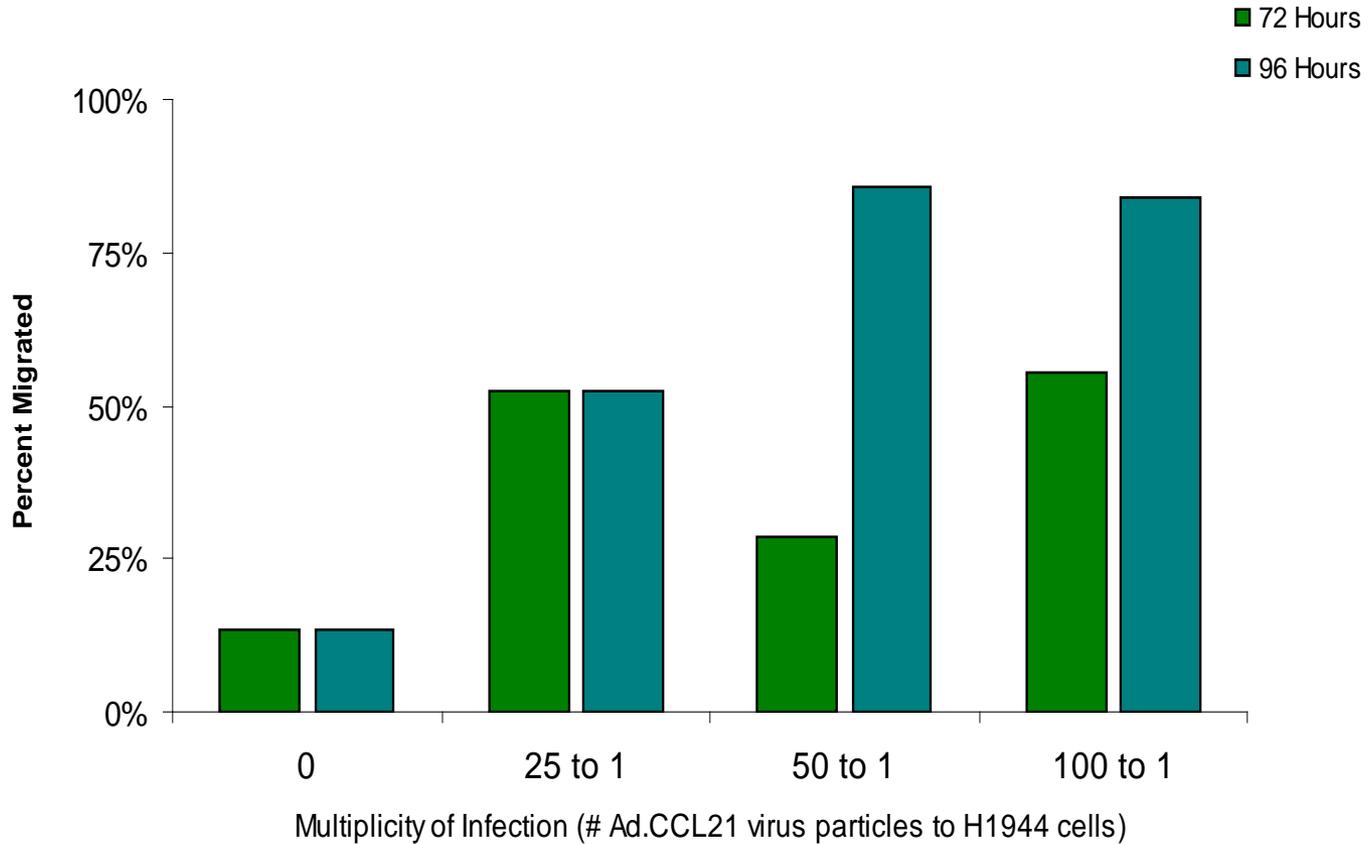
Transduction of CCL21 Chemokine-MOI

CCL21 secretion by NSCLC H1944 transduced with Ad.CCL21

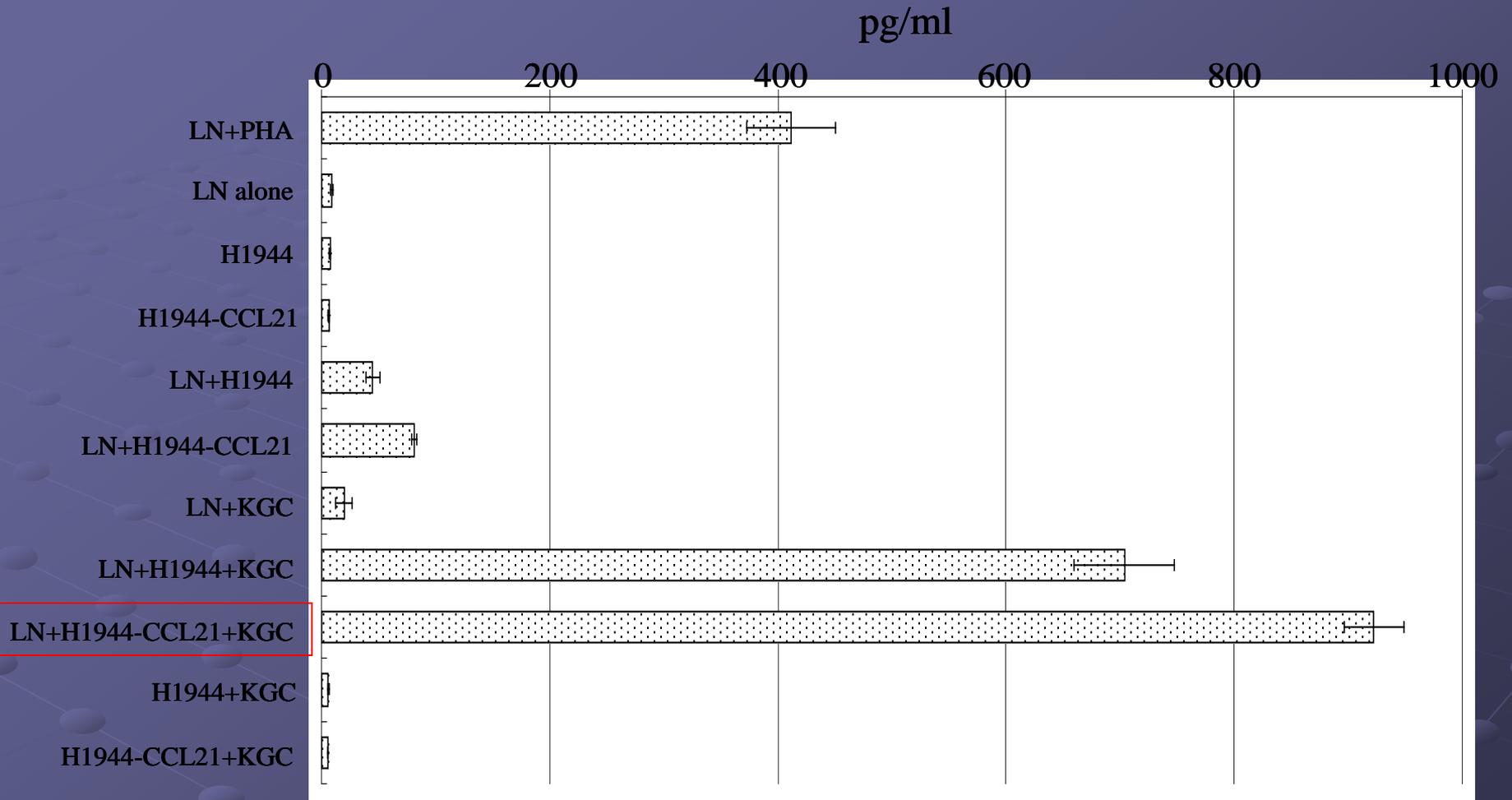


Chemotaxis Assay

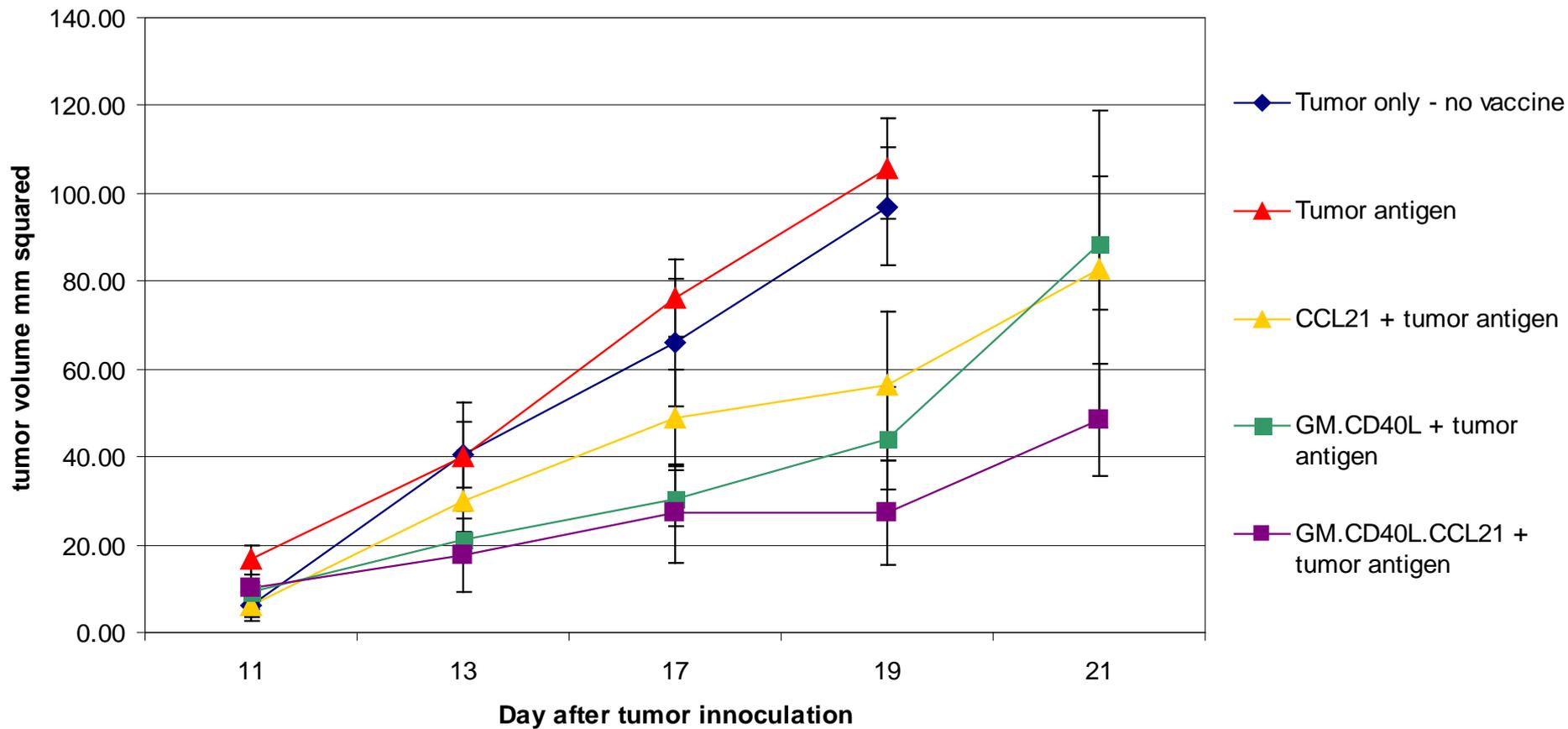
Migration of hT cells in Response to hCCL21 Secreted by Transduced H1944 Cells



IL-2 Production by LN Cells



CCL21 expression may augment anti-tumor immune responses induced by GM.CD40L. T cell-associated IL-2 secretion by LN cells can increase in the presence of H1944-derived CCL21 over untransduced H1944 tumor cells when cocultured with bystander cells.



Mice were inoculated with tumor cells on day 0 & vaccinated on D 7 mice, then 3 more times every 3-4 days. Tumor volume was measured. At the end of the study, lymph node cells and splenocytes were harvested. We did observe a significant increase in the time to tumor progression in all vaccine treated mice, however mice treated the GM.CD40L.CCL21 had a longer time to progression and an overall smaller tumor volume. P: 0.038

Clinical Studies with CCL21

Approved by the RAID and OBA programs

- A Phase I trial in subjects with advanced, chemotherapy naïve melanoma test the safety, immunologic activity, and potential efficacy of Ad-CCL-21-DC is underway.
 - Thusfar, 11 participants have been enrolled with no adverse events found. Immunoassays are underway
 - Unpublished, verbal conversation with Dr. Weber.
- At UCLA, a study is currently enrolling to a study entitled: *A Phase I Trial of CCL21 Gene Modified Dendritic Cells In Non-Small Cell Lung Cancer (NCT00601094)*.
 - Thusfar 6 patients with advanced NSCLC have been enrolled with no grade 3 or 4 adverse events
 - Verbal communication from Dr. Jay M. Lee- study PI.

Phase I/Phase II Study Flow

Adenocarcinoma
one prior chemo or >
ECOG 0-1
No Brain Metastases
Measurable Disease



Phase 1
Vaccine A

N: 3-6



MTD

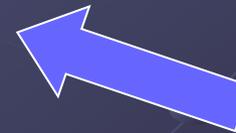


Vaccine A

Vaccine B

Phase II

N: 64



Vaccine A: GM.CD40L.CCL21
Vaccine B: GM.CD40L

Objectives

- ***Primary Objective***

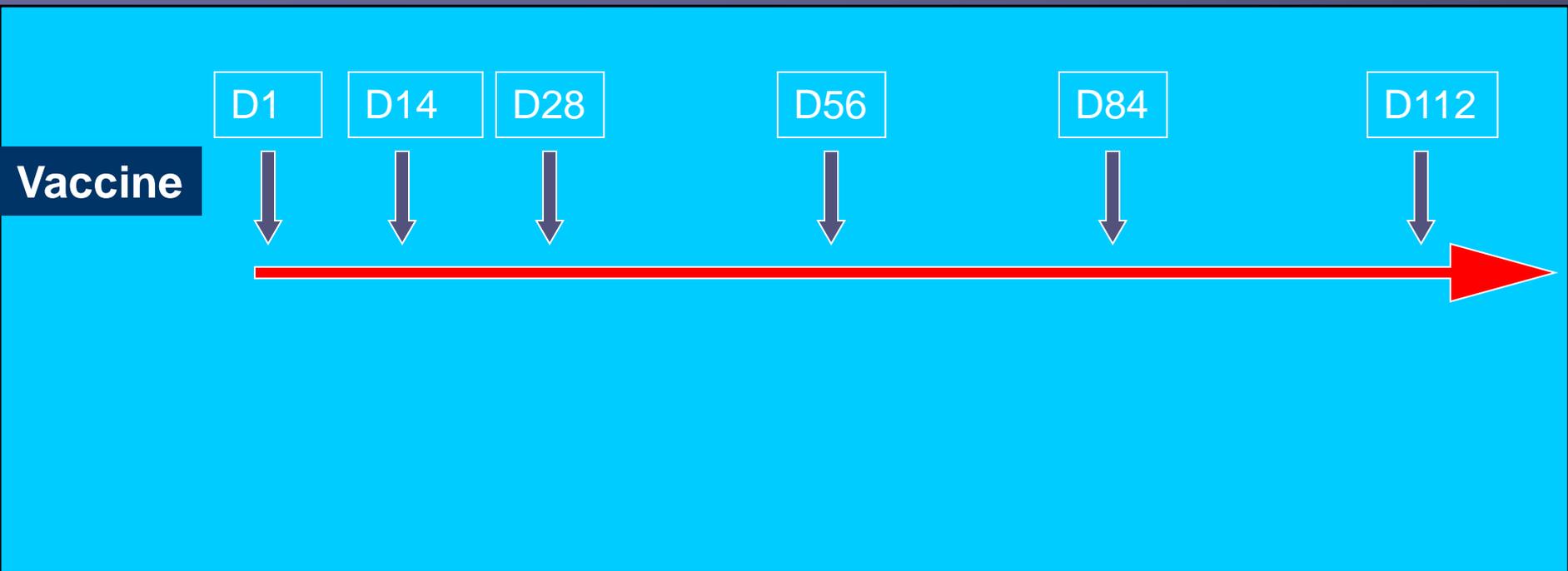
- To determine the safety and tolerability of GMCD40L.CCL21 vaccine
- To evaluate the 6 month progression free survival rate.

- ***Secondary Objectives***

- To evaluate the patients for the development of specific anti-tumor immune responses after immunization.
- To evaluate the survival of patients treated with the vaccine.
- To evaluate the tumor response rate

Vaccine Treatments

- Injected intradermally into 4 separate sites (0.25 ml injected at each site),
 - bilateral proximal upper (axillary nodal basins)
 - lower extremities (inguinal nodal basins)
- Vaccine A:
 - 7.5×10^6 irradiated H1944 tumor cells,
 - 7.5×10^6 irradiated H2122 cells,
 - 15×10^6 GM.CD40L cells (1.1 mL)
- Vaccine B:
 - Vaccine A
 - H1944 cells expressing CCL21



VACCINE SCHEDULE

Toxicity Assessments

- NCI Common Toxicity Criteria for Adverse Events (CTCAE), Version 4:
- DLT
 - Grade 4 neutropenia lasting >7 days in the absence of growth factor support
 - Grade 4 neutropenia associated with fever >38.5°C
 - Any other grade 4 hematological toxicity
 - Grade 3 or 4 nausea, vomiting or diarrhea despite prophylaxis or treatment with an optimal anti-emetic or anti-diarrhea regimen
 - Grade 2 immunologic toxicity (except fever)
 - Any other Grade 3 or higher non-hematological toxicity attributable to the study drug, excluding alopecia and fatigue.

Immune Testing

- Vaccine immunogenicity will be measured by *in vitro* testing of peripheral blood lymphocytes for cytokine-secreting T cells in ELISPOT assays.
- We will assess the WT1 and CEA (tumor antigens commonly expressed by NSCLC and our cell lines H1944 and H2122) specific T cell responses in the patients.

Outcomes Calculations

- Imaging studies (CXR, CT, MRI, or U/S, as indicated) will be obtained within 2 weeks before the beginning of protocol treatment, after the 3rd vaccine, and again 3 weeks after the sixth vaccine is administered.
- RECIST v 1.1

Statistical Considerations

- Phase I
- 3-6 patients will be treated on the phase I portion of the study.
- The CTCAE criteria will be used to grade toxicities, with a DLT defined in Section 11.1.
- No dose escalation is planned.

Statistical Considerations

● Phase II

- Once the MTD is established in the phase I, additional patients will be enrolled at this dose level to a total of 64 on a randomized phase II trial (32 per each arm).
- The primary endpoint for the trial will be 6-month progression free survival, PFS.
- For each arm using the two-stage Simon Minimax design with 10% type I error rate and 10% type II error rate, 18 patients will be enrolled in the first stage of the trial with 10% rejection error. If none of the patients are progression-free (i.e. all of them are either progressed or dead prior to 6 months), the treatment will be stopped. If 1 or more patients are progression-free, 14 additional patients (a total of 32 patients per group) will be enrolled. If a total number of progression-free subjects are greater than or equal to 4, the null hypothesis will be rejected.
- ESR for Toxicity and Lack of immunogenicity are also employed in this study

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