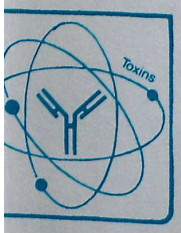
Volume 2, Number 1, 1989
ISSN: 0892-7049



ANTIBODY
mmunoconjugates, and
Radiopharmaceuticals

Editor: **STANLEY E. ORDER,** M.D., Sc.D., F.A.C.R.

ANTIBODY, IMMUNOCONJUGATES, AND
RADIOPHARMACEUTICALS
Volume 2, Number 2, 1989
Mary Ann Liebert, Inc., Publishers

Phase I Studies with a Murine Monoclonal Antibody Vinca Conjugate (KS1/4-DAVLB) in Patients with Adenocarcinoma

DENNIS SCHNECK,[1,2,3] FRED BUTLER,[3] WILLIAM DUGAN,[3]
DONNA LITTREL,[3] SUSAN DORRBECKER,[1] BRUCE PETERSON,[1]
RON BOWSHER,[1] ALLYN DELONG,[1] and JOHN ZIMMERMANN[1]

[1]*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN*
[2]*Departments of Medicine and Pharmacology, Indiana University School of Medicine, Indianapolis, IN*
[3]*Methodist Hospital, Indianapolis, IN*

ABSTRACT

The murine monoclonal antibody vinca conjugate (KS1/4-DAVLB) was infused as a single IV dose to 13 patients with colon or lung tumors. The dose ranged from 40 to 250 mg/M^2. Five patients also received KS1/4-[^{3}H] DAVLB. Nine patients were administered multiple IV doses of KS1/4-DAVLB. Dose limiting toxicity in both the single and multiple dose studies was acute onset of abdominal pain, nausea, emesis and diarrhea. The MTD in the single dose study was 250 mg/M^2 and a total cumulative dose of approximately 400 mg for most of the patients in the multiple dose study. Examination of duodenal biopsies in affected patients revealed ulceration of epithelium with loss of villous structure and intense acute inflammatory cell infiltrate. The degree of inflammation was dose-dependent. KS1/4-DAVLB bound extensively to duodenal epithelium. Complement activation on the surface of duodenal epithelium may be responsible for the acute inflammatory reaction. Significant localization of KS1/4-DAVLB to tumor cells was observed in the multiple dose study. KS1/4-DAVLB pharmacokinetic parameters were as follows: $t_{\frac{1}{2}}$ of 33 hr., distribution volume 4.9L and a clearance of 1.2 ml/hr/kg. Ten percent of the radioactive dose was recovered in the urine and 20% in the feces.

INTRODUCTION

Several studies have examined the potential therapeutic value of monoclonal antibody (moab) conjugates with radionuclides, plant and microbial toxins, and oncolytic agents such as methotrexate, adriamycin and the vinca alkaloids (1, 2, 3, 4). These efforts have sought to test the concept of utilizing the moab as a site-directed targeting agent to human tumors.

The murine (IgG2a) monoclonal antibody KS1/4 recognizes a tumor associated cell surface antigen which is a 40,000 M.W. glycoprotein found in high density on the tumor cell membrane of human lung, colon, rectal, pancreatic, and ovarian adenocarcinomas (5). The conjugate KS1/4-DAVLB containing 4-6 molecules of desacetylvinblastine covalently attached to KS1/4 via hemisuccinate linkers retains a high degree of reactivity with the tumor associated antigen. Preclinical pharmacology experiments related to the anti-tumor properties of

KS1/4-DAVLB have previously been reported (6). Preliminary results of Phase I studies utilizing KS1/4-DAVLB in patients with adenocarcinomas are reported in this communication.

METHODS

Patients

Thirteen patients with adenocarcinoma of the lung (stage III) colon or rectum (stage D) ranging in age from 40 to 76 years and in weight from 104 to 230 pounds participated in this study. Life expectancy of at least two months, and a Karnofsky performance value of at least 60% were required for enrollment. Adequate marrow function (Hb >10 g/dl, WBC >4,000/mm^3), liver function (bilirubin <2.5 mg/dl), and kidney function (creatinine <2.5 mg/dl) were also required. Nine patients ranging in age from 28 to 70 years and ranging in weight from 114 to 254 pounds and with metastatic lung, colon, or ovarian adenocarcinoma participated in the multiple dose study.

KS1/4-DAVLB was administered by the intravenous route over a time period not less than two hours. The infusion vehicle was normal saline containing human albumin (5 g/100 ml). A 1 mg test dose of KS1/4-DAVLB was administered to each patient prior to the main infusion. Adverse reactions to the test dose were not observed in any patient. The dose schedule for the patients administered a single infusion of KS1/4-DAVLB is shown in Table 1.

TABLE 1.

KS1/4-DAVLB Dose Schedule

Patient Number	KS1/4-DAVLB Dose (mg/M^2)	KS1/4-DAVLB Dose (mg)	Vinca Dose (mg)
1, 2	40	56, 60	1.4, 1.5
3, 4	80	168, 144	4.3, 3.6
5, 6	140	137, 175	3.5, 4.5
7*, 8*		196, 252	5.0, 6.4
9*		293	7.4
10*,11*	250	350, 500	8.9, 12.7
12, 13		525, 550	13.3, 13.9

Patients 6 and 11 had adenocarcinoma of the lung, the remainder had colorectal cancer.

* These patients received 100 µCi of [^{3}H]-labelled KS1/4-DAVLB.

The starting dose for each patient in the multiple dose study was 63 mg/M^2 administered every two or three days for up to nine doses.

Pharmacokinetic Studies

Serial blood samples were obtained from each patient who participated in the single dose study following KS1/4-DAVLB administration. The concentration of KS1/4-DAVLB in the serum prepared from these samples was measured by means of immunoradiometric (7) (IRMA) and flow cytometric (8) (FCM) assays. The initial binding reaction in the IRMA assay consists of the incubation of serum with beads coated with goat anti-mouse IgG antibody. The beads are washed and then further incubated with ^{125}I-labelled goat anti-mouse IgG antibody. The radioactivity adhering to the beads was quantified in a gamma counter. The murine IgG concentration in patient serum samples was estimated from a standard curve of KS1/4 in serum. The initial binding reaction in the FCM assay consists of serum samples incubated with P3-UCLA tumor cells which express the KS1/4 antigen. The mixture is then centrifuged, washed and the cell pellet resuspended and incubated with fluorescein conjugated sheep anti-mouse IgG antibodies. Aliquots of cells were analyzed for fluorescence intensity using an Epic's C flow cytometer. KS1/4-DAVLB serum concentrations were estimated from a standard curve developed from KS1/4 standards added to serum.

Aliquots of serum and urine from patients administered radioactive KS1/4-DAVLB were pipetted into Scintisol® and the radioactivity was determined in a Beckman LS 3801 liquid scintillation system. Aliquots of an aqueous fecal homogenate were combusted with a Packard Tri Carb Model B306 oxidizer. The radioactive content in the combusted samples was then measured in a manner similar to that described for the serum and urine samples. Urine and fecal outputs were measured daily for up to five days after infusion of radiolabelled KS1/4-DAVLB.

Assessment of Immune Responses (HAMA) to KS1/4-DAVLB Infusions

Blood was drawn at various time periods after infusions of KS1/4-DAVLB to obtain serum for measurement of concentrations of human anti-KS1/4-DAVLB antibodies.

The antibody response to KS1/4-DAVLB was measured utilizing an ELISA assay using microtiter plates with wells coated with the murine immunoglobulin KS1/4. Antibodies present in the serum react with KS1/4 coated on the surface of the wells. Binding of human antibodies to KS1/4 is detected by means of peroxidase conjugated goat anti-human immunoglobulins. The optical density of the well supernatents is measured following inculation with a perioxidase substrate system. The standard curve is constructed using serial dilutions of a monkey serum known to contain anti-KS1/4 antibodies.

Binding of KS1/4-DAVLB to Duodenal Epithelium and to Tumor Tissue

Freshly frozen duodenal biopsy samples were thawed and stained using direct or indirect standard avidin-biotin-complex (ABC) methods. Alternatively similar tissue sections were also stained using a standard 4 stage PAP method.

Binding of Complement to Duodenal Epithelium

Duodenal biopsy specimens were incubated with fluorescein labelled antibodies which interact with either the C1q or C3b components of complement. The sections were then examined utilizing a fluorescence microscope.

Safety Studies

Serial blood and urine samples were obtained from each patient following KS1/4-DAVLB infusion for measurement of CBC, biochemical profiles, and urinalysis. Special studies were performed when indicated. These studies included x-ray examinations of the upper gastrointestinal tract, panendoscopy and biopsy of the upper gastrointestinal tract, and colonoscopy.

Anti-Tumor Activity

When possible, direct measurements of accessible tumor tissue were made. Repeat x-rays and CT scans of tumor areas were performed. Serum CEA concentrations were repeated in a serial manner.

RESULTS

Eight of thirteen patients administered single doses of KS1/4-DAVLB developed symptoms of gastrointestinal tract toxicity. Seven of nine patients administered multiple doses of KS1/4-DAVLB also experienced the same type of toxic effect. These patients acutely developed nausea, vomiting, epigastric pain, and diarrhea. The symptoms were noted during or shortly after completion of the infusion. The maximum tolerated dose was 250 mg/M^2 in the single dose and approximately a total cumulative dose of 400 mg in the multiple dose study. Several patients had duodenal endoscopy at 24 to 52 hours after the start of the infusion and biopsies taken. The duodenal mucosa was observed to be edematous, congested, and friable. Microscopically loss of villous structure, epithelial cell degeneration, intense infiltration with polymorphonuclear and mononuclear cells, edema and vascular congestion were noted. The lesion was reversible and permament damage to the duodenum did not occur in any patient.

Two patients in the single dose study had endoscopy and duodenal biopsies at the onset of symptoms (approximately two hours after the start of the infusion). Gross and microscopic examination was normal at this time. Immunoperoxidase studies demonstrated significant binding of KS1/4-DAVLB to the epithelial cells. In addition, C1q and C3b deposition on the epithelium was observed. Repeat endoscopy of the duodenum at 24 hours in these patients revealed an edematous inflamed mucosa with acute inflammatory changes on microscopic examination. Intense binding of KS1/4-DAVLB to the epithelium and C1q and C3b deposition was also noted at this time.

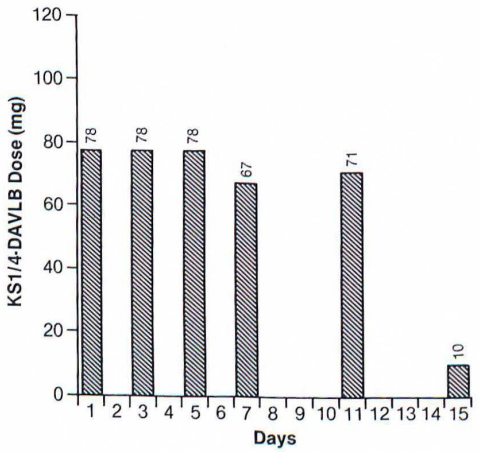


FIGURE 1. KS1/4-DAVLB Dose Schedule for a Patient Administered Multiple Doses

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.