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Monoclonal antibody drug conjugates for site-directed cancer chemotherapy: preclinical pharmacology and toxicology studies

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Introduction

Monoclonal antibodies (MoAbs) recognizing tumor-associated gene products offer a vehicle for site-directed therapy strategies for traditionally 'chemoresistant' human solid tumors. In theory, a MoAb could target chemotherapeutic agents to a tumor site sparing or limiting biodistribution of the oncolytic agent to normal proliferating tissues. This could achieve two goals: (a) less systemic toxicity to sensitive normal tissues and (b) increased oncolytic agent concentration at the desired tumor site. Both goals could contribute to an increased therapeutic index for a MoAb–oncolytic drug conjugate over conventional chemotherapy.

Efforts in these laboratories have focused on the combination of moabs with various vinca alkaloid species as covalent drug conjugates. Monoclonal antibody KS1/4, originally described as an antibody recognizing a human lung adenocarcinoma antigen (6), has been utilized in preclinical studies as a targeting vehicle for various human adenocarcinomas including lung and colorectal carcinomas. Two MoAb–drug conjugates: KS1/4-desacetyl-vinblastine (KS1/4-DAVLB, Lilly Serial # LY256787) and KS1/4-desacetyl-vinblastine-hydrazide (KS1/4-DAVLB-HY, Lilly Serial # LY203725) are being considered currently as human clinical trial candidates for the treatment of epithelial malignancies. This brief review will summarize aspects of the preclinical pharmacology and toxicology of these MoAb–drug conjugates which will be discussed in greater detail elsewhere (1–3, 5).

Results

KS1/4-DAVLB and KS1/4-DAVLB-HY represent two MoAb–vinca alkaloid conjugates utilizing unique conjugation chemistry strategies (1, 2, 4). These agents have been extensively characterized *in vitro* to optimize appropriate molar ratios of drugs/MoAb while maintaining excellent immunoreactivity toward target malignancies. Various biochemical and analytical profiles have also been employed which document the integrity of the antibody and the covalently attached vinca alkaloid species. *In vitro* analyses of the vinca alkaloid potency of vinblastine sulfate and desacetylvinblastine hydrazide suggest that these two related vinca alkaloid species are of similar activity. Comparable analyses of the KS1/4-DAVLB and KS1/4-DAVLB-HY conjugates by growth curve experiments of the P3-UCLA human lung adenocarcinoma cell line document that the KS1/4-DAVLB

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Table 1. Comparison of KS1/4-DAVLB-HY (LY203725) to KS1/4-DAVLB (LY256787): efficacy studies

Model	KS1/4-DAVLB-HY	KS1/4-DAVLB*
Tumor initiation model		
>50% Tumor growth suppression	0.0625	2.5
Established tumors		
Regression	0.25–0.50	10.0
Growth plateau	0.125	5.0
Experimental metastases		
For significant increase in survival	0.25	5.0

* Minimum effective dose (mg/kg) vinca.

conjugate is approximately two orders of magnitude less potent than vinblastine sulfate, whereas the KS1/4-DAVLB-HY conjugate is only slightly less potent than desacetyl-vinblastine hydrazide. These two conjugates of significantly different *in vitro* activity have been studied in various *in vivo* models of human tumors as xenografts in athymic nude mice. Three models with varied tumor burdens have been developed and applied to both human lung and colorectal xenograft studies. The initial system, termed the tumor initiation model, examines the efficacy of the conjugates and parallel free drug therapies (Rx i.v., days 2, 5, 8) on 48-h established subcutaneous xenografts in nude mice, representing a minimal tumor burden. The second system, termed the established tumor model, delays the onset of multiple i.v. Rx until the subcutaneous xenograft tumors have established for 16–20 days, often representing a tumor burden in the mouse from 2% to 4% of total body mass. In contrast to the first two systems, the third model establishes multifocal tissue metastases experimentally in the nude mouse providing the opportunity to do survival analyses rather than the measurement of the growth of a subcutaneous mass. Table 1 summarizes the results of approximately 80 *in vivo* experiments in which both the KS1/4-DAVLB conjugate and KS1/4-DAVLB-HY conjugates were employed in the above described models. As can be observed, both conjugates demonstrate efficacy in the various models but with significantly different potencies. Parallel studies to the KS1/4-DAVLB-HY conjugate with desacetylvinblastine hydrazide treatment groups, demonstrate that significantly higher doses of free drug (1.0–4.0 mg/kg) must be administered to achieve similar effects, i.e. in tumor initiation studies. However, in some models, the comparable efficacy of established tumors (for measured regression) and experimental metastases (for increase in survival) could not be achieved suggesting that the conjugated drug was superior to free drug administration on varied schedules. Studies parallel to the less potent KS1/4-DAVLB conjugate, with vinblastine sulfate treatment groups, revealed that similar doses of vinblastine in some cases could achieve comparable efficacy as the KS1/4-DAVLB conjugate but often not without severe toxicity. These data suggested that the toxicity of the vinca alkaloid species could be significantly altered by either more effective delivery of a potent oncolytic agent through a moab-conjugate (KS1/4-DAVLB-HY) or high dose effective delivery of a low potency oncolytic agent (KS1/4-DAVLB).

Toxicology studies and biodistribution studies were initiated to examine these two MoAb-drug conjugates and corresponding free vinca alkaloid species. Toxicology studies have been conducted in rats, athymic nude mice (for therapeutic index calculations from efficacy studies), and in Rhesus primates. Several of these studies are summarized in Table 2. These data document that both conjugates are less toxic than corresponding dosages of

Table 2. Toxicology of monoclonal antibody–vinca alkaloid conjugates

KS1/4-DAVLB/vinblastine	mg/kg
KS1/4-DAVLB LD10, LD_{50} (rats)	>13.0
Vinblastine LD_{50} (rats)	2.9 ± 1.5
KS1/4-DAVLB LD_{50} (athymic nude mice)	>25.0 (vinca content)
Vinblastine LD_{50} (athymic nude mice)	12.0
KS1/4-DAVLB (primates)	No toxicities to 2.0 mg/kg vinca content
Vinblastine (primates)	0.2 mg/kg induces toxicities (leucopenia)

KS1/4-DAVLB-HY/DAVLB-HY (pilot studies)	No effect dose (nonleucopenic) (mg/kg)
KS1/4-DAVLB-HY (rats)	1.5
DAVLB-HY (rats)	<0.5
KS1/4-DAVLB-HY (primates)	0.5
DAVLB-HY (primates)	0.1–0.25

vinca alkaloid in several species tested. The KS1/4-DAVLB conjugate does not demonstrate vinca alkaloid mediated toxicity in these studies at the doses indicated. In contrast, the KS1/4-DAVLB-HY conjugate does demonstrate vinca alkaloid related toxicities (leucopenia) at doses exceeding the no effect doses listed (>1.5 mg/kg in rats and >0.5 mg/kg in primates). These data document a correlation of higher *in vivo* efficacy/potency MoAb-drug conjugates with increased toxicological liability. Therapeutic index calculations [Toxic Dose ($>LD_{30}$)/Minimum Effective Dose (Efficacy)] on studies with KS1/4-DAVLB-HY conjugates demonstrate, however, that a range of 8.0–40.0 can be achieved in the various models described. In contrast, the range calculated for parallel free DAVLB-HY studies was from 0 to 4.0.

Biodistribution studies in tumor bearing athymic nude mice were also carried out on the KS1/4-DAVLB conjugate which had been labelled with a [^{3}H]-DAVLB moiety. These studies compared the pharmacokinetics and site-specific delivery of [^{3}H]-DAVLB to KS1/4-[^{3}H]-DAVLB in P3-UCLA human lung adenocarcinoma bearing nude mice and are described elsewhere in detail (5). A process of drug accumulation at the tumor site was found with a maximal concentration of vinca species attained with the KS1/4-DAVLB conjugate 96 h after dosing in tumor bearing nude mice. Up to 7–8% of the total administered dose was found in the tumor tissue after dosing with KS1/4-DAVLB. In contrast, less than 0.3% of the dose was found in tumor tissue after dosing with free DAVLB. In addition, the area under the curve of tumor tissue concentration versus time was about 2.7 orders of magnitude greater after dosing with KS1/4-DAVLB than with free DAVLB. Irrelevant (non-tumor reactive) MoAb–drug conjugates did not achieve tumor site-directed drug accumulation. Normal murine tissues with reticuloendothelial cell components (i.e. liver, spleen) were also sites of uptake of the KS1/4-DAVLB conjugate most likely mediated by Fc receptor catabolism of murine immunoglobulin. These data support directly the efficacy studies summarized above by providing direct evidence for site-directed vinca alkaloid localization to tumor tissue *in vivo*. The data also demonstrate that oncolytic drug concentration at a designated tumor site is greatly enhanced by the MoAb–drug conjugate as compared to conventional free drug administration.

Discussion

These data document that covalent drug–MoAb conjugates can demonstrate antitumor efficacy in preclinical models with decreased toxicity over conventional free drug administration. In one case, KS1/4-DAVLB, evidence has been discussed which documented that drug targeting had occurred to the tumor site *in vivo*. The KS1/4-DAVLB and KS1/4-DAVLB-HY conjugates provide two agents with different oncolytic potency to examine whether this site-directed strategy can be extended to human tumor therapy. Many theoretical and practical issues remain as potential obstacles to the success of this approach in human cancer therapy. These issues include:

(a) adequate potency of MoAb–drug conjugates, since conjugation can alter the drug's activity;

(b) adequate delivery of MoAb–drug conjugates to tumor sites in human, high percentage injected doses, as recorded as localized to tumors in athymic nude mice xenograft studies, may not be apparent in human trials;

(c) tumor target heterogeneity in antigen expression, as well as sensitivity to the delivered oncolytic agent;

(d) innocent bystander tissue toxicology as MoAbs also recognize similar antigens on select normal tissues, and

(e) the immunogenicity of murine monoclonal antibodies in the human host with repeated injections.

The importance of these issues in multiple dose therapy of human solid tumors with MoAb–drug conjugates such as KS1/4-DAVLB and KS1/4-DAVLB-HY will be discovered as Phase 1 human clinical trials begin soon on the initially developed monoclonal antibody–drug conjugates. Strategically, basic research efforts are in place to address potential antibody, drug potency and immunogenicity issues. These programs will be prioritized by early clinical results.

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