Clinical Development of 17-Allylamino, 17-Demethoxygeldanamycin

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Abstract: 17-allylamino, 17-demethoxygeldanamycin (17AAG; NSC 330507) is the first modulator of heat shock protein 90 (Hsp90) to enter clinical trials. Hsp90 serves a chaperone role to properly fold and deliver client proteins to appropriate intracellular locations. Interest in Hsp90 modulators for the experimental therapeutics of cancer has arisen based on pre-clinical evaluations suggesting that Hsp90 client proteins regulate signaling pathways critical to the molecular economy of many types of tumors, including oncogene signaling, cyclin-dependent kinase activation, steroid hormone receptors, and mediators of invasion and metastasis. Thus, Hsp90-directed agents could affect molecules upon which tumors depend for their proliferation and survival. Initial clinical studies have therefore sought to incorporate assessment of these endpoints into initial clinical evaluations. Three schedules of administration have been supported for initial evaluation in Phase I studies sponsored by the National Cancer Institute (NCI) or supported by NCI and sponsored by Cancer Research UK. In the daily times five schedule, a recommended Phase II dose (RPTD) of 40 mg/m^2 has been reached, while once weekly or three of four weekly schedules are defining RPTDs of 295 and 308 mg/m^2. Toxicity is tolerable and appears dominated by hepatic, gastrointestinal, and constitutional symptoms. Concentrations of drug at peak of ~1700-3000 nM are concordant with concentrations predictive of useful outcomes in pre-clinical model systems. Evidence of modulation of Hsp90 partner molecules has been obtained in both surrogate and some tumor compartments. These very early results encourage additional clinical evaluations of 17AAG and related molecules.

INTRODUCTION

This update will provide the current status of clinical activities with 17-allylamino, 17-demethoxy geldanamycin[1] (17-AAG) as of December 2002. The drug was originally defined [1] as a geldanamycin analog with evidence of *in vitro* anti-proliferative activity at concentrations where destabilization of Hsp90 chaperone partners such as the p185 c-*erb*B2 protein tyrosine kinase could be documented [2]. Further studies focused on comparing its potential for toxicity in comparison to the parent compound [3].

PRE-CLINICAL TOXICITY STUDIES

The toxicity of 17-AAG was evaluated pre-clinically in rats and dogs. In rats, geldanamycin produced severe hepatotoxicity and mortality at doses < 30 mg/M^2; whereas doses up to 75 mg/M^2 of 17-AAG were well tolerated and renal toxicity appeared to be dose limiting. In dogs, a single one-hour infusion of up to 500 mg/M^2 of 17-AAG was well tolerated, whereas a single one-hour infusion of 100 mg/M^2 of geldanamycin was lethal. Gastrointestinal (GI) toxicity appeared to be dose limiting for 17-AAG and hepatotoxicity appeared to be dose limiting for geldanamycin in dogs. The hepatotoxicity of geldanamycin was characterized clinically by elevations in liver transaminases and microscopically as hepatic and bile duct necrosis and bile duct hyperplasia in both species. Thus, in single dose intravenous pilot studies in both species, 17-AAG was found to be markedly less toxic compared to the parent compound, geldanamycin.[2]

There was no schedule dependent toxicity for 17-AAG since frequency of dosing (once or twice a day for 5 days) did not change the daily maximal tolerated dose (MTD) in either species.[3] The MTD was between 100-200 mg/M^2/day in dogs and 120-180 mg/M^2/day in rats using both schedules in range-finding studies. In addition, the single dose MTD in dogs was similar to the maximum tolerated total dose given over 5 days. The toxicity profile after multiple daily doses of 17-AAG was somewhat different than that seen after a single dose with the occurrence of gall bladder and hepatobiliary toxicity in addition to GI toxicity in a dose-related manner. This was manifested by increases in liver transaminases, bile acids, γ-glutamyl transpeptidase (GGT) and bilirubin in association with bile duct hyperplasia in the liver; together with hemorrhage, inflammation and necrosis in the common bile duct and gall bladder.

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[1] Note that NSC 330507's proper chemical name and the compound actually studied here is 17-allylamino, 17-demethoxygeldanamycin, abbreviated 17-AAG, although literature citations, including *vide infra*, have erroneously called it 17-allylaminogeldanamycin.

[2] Page, J.; Heath, J.; Fulton, R.; Yalkowsky, E.; Tabibi, E.; Tomaszewski, J.E.; Smith, A.; Rodman, L. Comparison of Geldanamycin (NSC-122750 and 17-Allylaminogeldanamycin (NSC-330507) Toxicity in Rats. *Proc. Amer. Assoc. Cancer Res*., **1997**, *38*, 308, [Abstract No. 2067].; Noker, P.E.; Thompson, R.B.; Smith, A.C.; Tomaszewski, J.E.; Page J.G. Toxicity and Pharmacokinetics of 17-Allylaminogeldanamycin (17-AAG, NSC 330507) in Dogs. *Proc. Amer. Assoc. Cancer Res.,* **1999**, *40*, 121, [Abstract No. 804].

[3] Page, J. G.; Noker, P. E.; Tomaszewski, J. E.; Smith, A. C. Lack of Schedule Dependent Toxicity of 17-allylaminogeldanamycin (17-AAG, NSC 330507) in Rats. *Proc. Amer. Assoc. Cancer Res*. **1999**, *40*, 121, [Abstract No. 805].

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Plasma levels determined at the end of the infusion that were associated with the MTD in dogs ranged from 2500-4900 nM. The recommended starting dose of 10 mg/M^2/day, given as a 1-hour IV infusion once a day for 5 days, was based on the dog (1/10 the MTD in the more sensitive species). When 10 mg/m^2/day of 17-AAG in the DMSO formulation was tested in rats, no clinical or histopathological adverse events were observed.

Initial Clinical Goals

Hsp90 modulators may require prolonged treatment intervals. They are known to modulate chaperone protein interactions with many client proteins, such as p185^{erb}B2. Alternate endpoints, such as receptor turnover or decreased oncogene product expression in tumor (xenograft) tissues, in conjunction with prolonged time to progression (cytostasis), may therefore be appropriate initial measures in early trials of the anticancer activity of such molecules [4]. For these reasons, in addition to the usual Phase 1 trial endpoints defining the MTD, attendant dose limiting toxicity (DLT), and clinical pharmacology parameters, biological markers of drug effect were incorporated into early clinical trials. These have included assessments of the level and function of Hsp90 partners such as the androgen, estrogen, glucocorticoid and epidermal growth factor receptors, raf-1, p56^{lck}, Akt, and p53, in addition to heat shock proteins 70, 72, and 90, as an integral part of NCI-sponsored Phase I trials of 17-AAG. In some cases these are being assessed in surrogate tissues such as peripheral blood mononuclear cells (PBMC), and where possible in biopsies of tumor tissue. When tumor tissue is obtained, in addition to the above markers, intra-tumoral levels of tumor DT-diaphorase, the product of the NQO1 gene with its attendant polymorphisms will also be of importance to track [5].

CLINICAL TRIALS WITH 17-AAG

Two trials of the daily times 5-day regimen and two trials of day 1, 8, and 15 administration, repeated every 28 days were initially undertaken. In addition, a study of a single dose regimen was supported by NCI in the United Kingdom in collaboration with Cancer Research UK.

In the UK study (T99-0013), conducted by Dr. Ian Judson, the starting dose was 10 mg/m^2 administered as a 15- to 30-minute IV infusion once every four weeks. Dose doubling occurred until drug-related grade 1 renal, hepatic, or cardiac toxicity or two episodes of grade 2 toxicity was observed, following which dose escalation increments of 40% were employed. By May 2002, the starting dose of 10 mg/m^2 had been escalated to 450 mg/M^2, and 22 patients treated[4] Doses were doubled until the 320 mg/M^2 dose was reached, after which dose escalation was in 40% increments. Two patients have had stable disease for more than 3 months. Two of six patients treated at 320 mg/M^2 had grade 3 nausea and vomiting. One patient at the 320 mg/M^2 dose level experienced a grade 3 peri-rectal bleeding and diarrhea leading to cardiovascular collapse that required hospitalization and was reported as possibly drug-related but was more likely due to dehydration and other factors, as there was a prior history of peri-rectal bleeding and hemorrhoids.

A Phase I evaluation of 17-AAG administered as a 1-hour IV infusion daily for 5 every 3 weeks (T98-0075) was activated in June 1999 at the NCI/ National Naval Medical Center, Bethesda, conducted by Dr. Jean Grem. In this study, the starting dose was also 10 mg/M^2, and dose has been escalated in 40% increments to 40 mg/M^2/day using the accelerated dose-titration scheme developed by Simon *et al.* [6]. One patient is treated per dose level until either one patient exhibits cycle 1 DLT, or two patients experience grade 2 drug-related toxicity during their first cycle at any dose level. Once either of these events occur, the accelerated dose titration scheme is discontinued, additional patients are entered at the dose level that triggered the change, and the study design reverts to a standard Phase I dose-escalation design using 40% increments. In June 2000, the protocol was amended to exclude patients with greater than 50% tumor involvement of liver, regardless of the degree of alteration of liver function tests, as the toxicity profile was mainly hepatic. Early data on 28 patients treated on this study[5] using an accelerated dose titration design reported that the accelerated dose escalation was employed until the 28-mg/M^2 dose level was reached. Standard cohort expansion was used at the next two dose levels, 40 mg/M^2 and 56 mg/M^2, respectively. Dose-limiting reversible grade 3 hepatotoxicity occurred at the 56-mg/M^2 dose level. The MTD and recommended Phase II dose on this schedule is 40 mg/M^2, a dose at which altered Hsp90 function is observed in PBMC. The toxicities experienced by patients included fever, emesis, anemia, and fatigue. The schedule was changed by amendment in August 2001 to a biweekly (every other week) schedule with a starting dose of 40 mg/M^2/day to determine if clinical activity and client protein modulation could be observed.

A Phase I trial of 17-AAG also on a daily × 5, administered as a 1 hr IV infusion every 21 days schedule (T99-0035), was activated in July 1999 at Memorial Sloan-Kettering Cancer Center and is being conducted by Dr. Howard Scher. The starting dose was 5 mg/m^2/day with an accelerated dose escalation using 100% increases in dose increment until a patient experiences one DLT (grade 3 or 4 toxicity) in the first course of treatment or until two grade 2 toxicities in two different patients are observed in any course. Escalation is then continued in 40% increments using a standard Phase I design. Toxicity information on the

[4] Banerji, U.; O'Donnell, A.; Scurr, M.; Benson, C.; Hanwell, J.; Clark, S.; Raynaud, F.; Turner, A.; Walton, M.; Workman, P.; Judson, I. Phase I Trial of the Heat Shock Protein 90 (HSP90) Inhibitor 17-allylamino 17-demethoxygeldanamycin (17AAG). Pharmacokinetic (PK) Profile and Pharmacodynamic (PD) Endpoints. *Proc. Am. Soc. Clin. Oncol.* **2001**, *20,* 82a, [Abstract 326]; Banerji, U.; O`Donnell, A.; Scurr, M.; Benson, C.; Brock, C.;Hanwell, J.; Stapleton, S.; Raynaud, F.; Simmons, L.; Turner, A.; Walton, M.; Workman, P.; Judson, I. *et al.* (**2002**). A Pharmacokinetically (PK) - Pharmacodynamically (PD) Driven Phase I Trial of the Hsp90 Molecular Chaperone Inhibitor 17-allyamino 17-Demethoxygeldanamycin (17AAG). *Proc. Am. Assoc. Cancer Res.***2002**, *43*, 272, [Abstract 1352].

[5] Wilson, R. H.; Takimoto, C.H.; Agnew, E.B.; Morrison, G.; Grollman, F.; Thomas, R.R.; Saif, M. W.; Hopkins, J.; Allegra, C.; Grochow, L.; Szabo, E.; Hamilton, J. M.; Monahan, B. P.; Neckers, L.; Grem, J. Phase I Pharmacologic Study of 17-(Allylamino)-17-demethoxygeldanamycin (AAG) in Adult Patients with Advanced Solid Tumors. *Proc. Am. Soc. Clin. Oncol.* **2001**, *20*, 82a, [Abstract 325].

Table 1. Summary of the Agent Specific Toxicities Encounted in Various Clinical Trials

Category	Adverse Events
Blood/Bone Marrow	Hemoglobin
Constitutional Symptoms	Fatigue (lethargy, malaise, asthenia) Anorexia
Gastrointestinal	Dehydration Diarrhea patients with a colostomy Diarrhea patients without colostomy Nausea Vomiting
Hepatic	Alkaline phosphatase GGT (Gamma-Glutamyl transpeptidase) SGOT (AST) serum glutamic oxaloacetic transaminase) SGPT (ALT) (serum glutamic pyruvic transaminase)
Pain	Myalgia (muscle pain)

first 16 patients has been published.[6] At 80 mg/M^2, DLTs were diarrhea, thrombocytopenia, and transient transaminitis. Dose escalation was switched from the accelerated phase to the standard phase. Two patients experienced DLTs on day 4 and 5. Subsequently, three patients each were studied at 40 mg/M^2 and 56 mg/M^2 with no significant adverse events. In patients evaluable for response, stable disease was reported in 4/13 patients beyond 3 months duration. The protocol was amended in January 2001, to treat patients for 3 consecutive days instead of 5, with cycles repeated every 14 days instead of every 21 days.

A Phase I trial of 17-AAG administered on an intermittent schedule (days 1, 8, and 15 on a 28-day cycle) by 1-hour IV infusion in solid tumor patients (T99-0058) was activated in August 2000 and is being conducted by Dr. Charles Erlichman[7] at the Mayo Clinic. 17-AAG was administered at a starting dose of 15 mg/M^2. The same accelerated dose escalation scheme described previously for T98-0075 (40% dose increments) is being used in this study. Dose escalation reached 112 mg/M^2 without any significant toxicity occurring. An effect on only one biomarker was observed; therefore, the protocol was amended in January 2001 to continue the accelerated phase until either DLT or moderate toxicity occurs. In March 2001, preliminary data on eight patients was presented. The most common but non-dose limiting toxicities have been anemia, anorexia, diarrhea, nausea, and vomiting. Dose escalation has reached 431 mg/M^2, and the only toxicities above grade 3 occurred in one patient at 308 mg/M^2, who died from pulmonary causes. Although the death was probably due to pre-existing pulmonary disease, a relationship to 17-AAG could not be excluded. At 431 mg/M^2, grade 3 anorexia, nausea, vomiting, anemia, and dehydration were reported in one patient. The recommended phase two dose on the schedule was 308 mg/M^2. The protocol was amended in August 2000 to require administration of the drug in the General Clinical Research Center, because the DMSO odor from the vehicle was offensive to others around the patient. The protocol was amended in February 2001 to add a second cohort in whom patients will be treated on a biweekly × 3 every 3 weeks schedule with a starting dose of 57 mg/m^2. Both cohorts will be dose-escalated to an MTD.

An additional Phase I trial of 17-AAG on the intermittent 1-hour IV infusion on days 1, 8, and 15 every 28 days was activated in October 1999 at the University of Pittsburgh and is being conducted by Dr. Ramesh Ramanathan (T99-0038). Eight patients were treated with the starting dose of 10 mg/M^2. Only one drug-related grade 2 toxicity, alopecia, was reported in the first three patients; however, additional patients were accrued at this dose level because one patient developed cardiac tamponade (which was subsequently assessed as unlikely to be drug-related). Grade 3 elevated transaminase was reported in the third patient treated at 55 mg/M^2, but only grade 1 adverse events were reported in the two additional patients entered at that dose level. Dose escalation had only reached 127 mg/M^2 on this trial that uses a standard 3-6 patient per dose level escalation scheme at a time when the Mayo group had exceeded 220 mg/M^2 treating patients on the same schedule, but using an accelerated dose escalation scheme.

Consequently, this protocol was amended in June 2001 to enroll the next cohort at 220 mg/M^2. A grade 3 hypertension in one patient treated at 295 mg/m^2 was considered possibly drug-related. The recommended phase two dose in this schedule was 295 mg/M^2. The protocol was subsequently amended to explore a twice-weekly dose administration schedule for 3 of every 4 weeks.

Safety Information from Clinical Trials

A summary of the agent specific toxicities coded in various trials is presented according to organ system in Table 1. On the daily × 5 schedule, reversible hepatotoxicity was the DLT in protocol T98-0075 at 56 mg/M^2. In an attempt at further dose escalation, protocol T99-0035 (daily × 5 schedule every 3 weeks) was amended to treat patients on a daily × 3 every 14 days schedule. The DLTs were diarrhea, thrombocytopenia, and transient transaminitis at 80 mg/M^2, and dose escalation was switched from an accelerated to a standard phase. The elevated liver function

[6] Munster, P. N.; Tong, W., Schwartz, L.; Larson, S.; Kenneson, K.; De La Cruz, A.; Rosen, N.; Scher, H.; Phase I Trial of 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) in Patients (Pts) with Advanced Solid Malignancies. *Proc Am Soc Clin Oncol.* **2001**, *20,* 83a [Abstract 327].

[7] Erlichman, C.; Toft, D.; Reid, J.; Sloan, J.; Atherton, P.; Adjei, A.; Ames, M.; Croghan, G. A Phase I Trial of 17-Allyl-amino-geldanamycin in Patients With Advanced Cancer. *Proc. Am. Assoc. Cancer Res.***2001**, *42*, 833, [Abstract 4474].

values were not unexpected because liver toxicity was one of the primary toxicities produced by 17-AAG in rats and dogs. Other grade 2 or higher toxicities that occurred in more than one patient were elevated GGT and metabolic (hyperglycemia). Grade 1 transient numbness and tingling in the fingers and toes was reported in one patient. On the weekly dose administration schedule for protocol T99-0058, dose escalation has reached 431 mg/M^2. The only toxicities above grade 3 occurred in one patient at 308 mg/M^2, who died from pulmonary causes. The death was probably due to pre-existing pulmonary disease, but a relationship to 17-AAG could not be excluded. At 431 mg/M^2, grade 3 anorexia, nausea, vomiting, anemia, and dehydration were reported in one patient. The most common, but non-dose limiting toxicities have been anemia, anorexia, diarrhea, nausea, and vomiting. On protocol T99-0038, one of three patients treated at 55 mg/M^2 had a grade 3 elevated transaminases. The only other grade 3 or higher adverse event considered possibly drug-related on this trial was a grade 3 hypertension in one patient treated at 295 mg/M^2.

Because 17-AAG is formulated using egg phospholipids, patients with a history of serious allergic reactions to eggs should not receive this agent. Studies suggest that 17-AAG is metabolized by CYP3A4. Therefore, patients who are taking agents that alter CYP3A4 activity (e.g., grapefruit juice, ketoconazole, fluconazole, itraconazole, cyclosporine, erythromycin, clarithromycin, cimetidine, terfenadine, astemizole, and/or the HIV protease inhibitors indinavir and nelfinavir) should not receive this drug [7,8].

Clinical Pharmacokinetics and Pharmacodynamics

The pharmacokinetics of 17-AAG in NCI-sponsored or supported clinical trials are reviewed here and summarized in Table 2. Previous studies had indicated that 17-AAG can be metabolized to 17-amino, 17-demethoxygeldanamycin (17-AG), which does possess anti-proliferative activity [9]. Therefore in addition to measuring 17-AAG in the initial clinical studies, generation of 17-AG was a matter of interest to consider in the clinical setting, as it might be correlated

Table 2. Summary of AG and 17-AAG Clinical Pharmacokinetic Parameters

Protocol / schedule/ dose	Cmax (nM)	t1/2 (hr)	CL	AUC (nM•hr)	Vd (L/m^2)	Comments	Ref
T98-0075 Daily × 5 q 3 weeks							
40 mg/m^2	AAG 1860 ± 660	(terminal) 2.5 ± 0.5	41 ± 13.5 L/hr	-	86.6 ±34.6	Recommended Phase I[I] dose = 40mg/m^2; C_{max} & AUC ↑ proportionately with dose; CL remained constant; d1 vs. d5 values not significantly different	Wilson *et al.,* 2001 (Footnote 5)
56 mg/m^2/day	AAG 2080	(terminal) 3.8	19.9 L/hr/m^2	6708	92	MTD = 40 g/m^2/day	Agnew *et al.*,. 2002 (Footnote 8)
	AG 770	(terminal) 8.6	30.8 L/hr/m^2	5558	203		
T99-0035 Daily × 5 q 3 weeks							
80 mg/m^2	AAG 2700	1.5	-	-	-	C_{max} values exceeded the 10-500 Nm needed for cell cytotoxicity	Munster *et al.,* 2001 (Footnote 6)
	AG 607	1.75	-	-	-		
T98-0058 d1, 8, 15 q 3 weeks							
15-157 mg/m^2	AAG -	(median) 2.3	(median) 26.6 L/hr/m^2 (444 mL/min/m^2)	-	-	C_{max} increased linearly with dose; AG detected at all dose levels	Erlichman *et al.,* 2001 (Footnote 7)
	AG -	(median) 4.6	-	-	-		
T99-0013 – non-DCTD-sponsored trial Once q week							
10-450 mg/m^2/wk	AAG -	-	(mean) 47.3 L/hr	-	(mean) 186 L	AUC ↑ linearly with dose; significant levels of AG were detected; plasma levels over 120 nM were maintained for more than 24 hours at the 450 mg/m^2 dose level	Banerji *et al.*, 2002 (Footnote 4)
450 mg/m^2/wk	AAG 16710	-	-	-	-		

with toxicity as well as allowing an additional means of conveying an anti-tumor effect. In the once weekly protocol T99-0013. The area under the concentration x time curve (AUC) increased in a dose dependent and linear fashion (R^2 = 0.757). The volume of distribution (V_d)was 186 L and the clearance was 47.3 L/hr. The maximal plasma concentration (C_{max}) at 450 mg/M^2 was 16800 nM. Concentrations over 120 nM, the median IC_{50} of the 60-cell line NCI panel, were observed for at least 24 hours, as was the active metabolite 17-AG. At the highest dose (450 mg/M^2/week) the pharmacodynamic markers in peripheral blood lymphocytes, showed a reduction in the expression of raf-1 and cdk4 between 24-48 hours, as well as induction of Hsp70 which had not returned to baseline at 96 hours. Tumor biopsies were performed before treatment and 24-hours post-treatment in four patients (three at 320 mg/M^2/week and one at 450 mg/M^2/week). A comparison of pre- and post-treatment tumor biopsies showed an induction of Hsp70 in all of four post-treatment samples, cdk4 depletion in 3/4 samples, and raf-1 depletion in one of four samples. Ongoing studies are now evaluating paired tumor biopsy specimens at 48 hours and may be extended further depending on the results of the pharmacodynamic studies.

Preliminary pharmacokinetics were evaluated in patients on the T98-0075 protocol at the NCI-National Naval Medical Center. The drug was given as a 1-hour infusion daily × 5 every 3 weeks, and C_{max}, AUC, terminal half-life, clearance (Cl), and V_d were compared using a non-compartmental analysis for 17-AAG and 17-AG at 56 mg/M^2. [8] The C_{max} values obtained for 17-AAG and 17-AG were 2080 nM and 770 nM, and the AUC values were 6708 and 5558 nM·hr, respectively. The terminal half-life of 17-AAG was shorter than that of 17-AG, 3.8 versus 8.6 hr, respectively. The Cl of 17-AAG and 17-AG was 19.9 and 30.8 L/hr/m^2, and the V_d were 92 and 203 L/m^2, respectively. For all dose levels, the total amount of drug recovered in the urine over a 72-hour period was 10.6% for 17-AAG and 7.8% for 17-AG. No significant differences between the day 1 and day 5 pharmacokinetic values were noted. This result was concordant with pre-clincal observations of prominent biliary excretion of 17-AAG[9]. The C_{max} and AUC tend to increase with increasing dose. No change in clearance was reported. Peak plasma concentrations ranged from 530 to 1300 nM in three patients who received doses of 10, 14, and 20 mg/M^2 to 1860 ± 660 nM at 40 mg/m^2 and 3170 ± 1310 nM at 56 mg/m^2. At 40 mg/m^2 (the MTD and recommended Phase I^I dose), the mean ± SD values for terminal half-life, Cl, and V_{dss} were 2.5 ± 0.5 hours, 41.0 ±13.5 L/hour, and 86.6 ± 34.6 L/m^2, respectively. A two-compartment, open model best fit the PK data.

In protocol T99-0035 performed at the Memorial Sloan Kettering Cancer Center, 17-AAG was administered daily for 5 days by IV infusion every 3 weeks. The half-life of an 80 mg/M^2 dose was 1.5 hours, and the peak plasma concentration was 2700 nM at 30 minutes. 17-AAG plasma levels at 1, 6, 24, 48, 72, and 96 hours were 1830 nM, 193 nM, 36 nM, 51 nM, 63 nM, and 57 nM, respectively. 17-AG was also measured; the half-life was 1.75 hours, and the peak plasma level was 607 nM at 1 hour. 17-AG plasma levels at 0.5, 6, 24, 48, 72, and 96 hours were 262 nM, 138 nM, 46 nM, 72 nM, 101 nM, and 39 nM.

The Mayo Clinic reported preliminary pharmacokinetic data for protocol T99-0058, a Phase I trial of 17-AAG given on days 1, 8, and 15 every 4 weeks. The median clearance of plasma samples (n=7) drawn on day 1 was 444 (range 208-4885) mL/min/M^2. C_{max} increased linearly with dose, and the half-life was 138 minutes. The half-life of 17-AG was 277 minutes.

FUTURE DEVELOPMENT

Once a recommended Phase II dose and schedule are established, the NCI plans to pursue relatively broad based clinical trials, reflecting the interest of the investigator community. The recommended Phase II dose may be modified based on observed persistent modulation of client proteins in disease specific malignancies. A current update of recent modifications to the trials described here is presented in Table 3. The diseases targeted for Phase II studies with 17-AAG will be chosen based on a growing body of pre-clinical data [5, 10-14] in for example, breast, prostate and lung cancers, certain leukemias[10] including chronic myelogenous leukemia (CML) [15] and lymphomas [16], as well as response data emerging in the Phase I investigations combined with pre-clincal observations.

Phase I combination studies driven by several pivotal pre-clinical observations have been undertaken early in the development of 17-AAG because its anti-tumor effect may be cytostatic in nature and thus used to best effect in combinations where their modulator activity on client protein levels might augment the activity of conventional cytotoxic agents, or other signal transduction inhibitors. Specifically, combinations of 17-AAG with taxanes for eventual treatment of breast, prostate, and lung cancers [17-19] have been described, as have favorable combination with radiation[11] and with imatinib mesylate for CML[12]. It is

[8] Agnew, E. B.; Neckers, L. M.; Hehman, H. E.; Morrison, G.; Hamilton, J.M.; Monohan, B. P.; Grem, J.L.; Takimoto, C.H. Human Plasma Pharmacokinetics of the Novel Antitumor Agent, 17-Allylaminogeldanamycin (AAG) Using a New HPLC-Based Analytic Assay. *Proc. Am. Assoc. Cancer Res.* **2000**, *41*, 701, [Abstract 4458]; Agnew, E. R.; Wilson, R.H.; Morrison, G.; Neckers, L.M.; Takimoto, C.H.; Grem, J.L. Clinical Pharmacokinetics of 17-(Allylamino)-17-demethoxygeldanamycin and the Active Metabolite 17-(Amino)-17-demethoxygeldanamycin Given as a One-Hour Infusion Daily for 5 Days. *Proc. Am. Assoc. Cancer Res.***2002**, *43*, 272, [Abstract 1349].

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[10] Yao, Q.; Hudson, W.; Taylor; M.; Kersey, J.H. Specific Inhibition of MLL Fusion Gene Leukemias by the Heat Shock Protein Inhibitors Herbimycin A and 17-Allylamino-17-demethoxygeldanamycin. *Proc. Am. Assoc. Cancer Res*. **2002**, *43*, 923, [Abstract 4576].

[11] Enmon Jr. R.; Yang, W-H, ; Solit, D.B.; Ballangrud, A.M.; Rosen, N.; Scher, H. I.; Sgouros, G. Synergistic Interaction of a Geldanamycin Analog (17AAG) and Radiation in Human Prostate Tumor Spheroids. *Proc. Am. Assoc. Cancer Res,* **2002**. *43,* 130, [Abstract 655].

[12] Bhalla, K.N.; Nimmanapalli, R.; O'Bryan, E. 17-Allylamino-17-demethoxygeldanamycin (17-AAG) Lowers Bcr-Abl Levels and Induces Differentiation and Apoptosis of STI571 Sensitive and Resistant Bcr-Abl Positive Acute Leukemia Cells. *Proc. Am. Assoc. Cancer Res.* **2001**, *42* , 800, [Abstract 4293].

Table 3. NCI- and Non-NCI Sponsored Clinical Trials with 17-AAG

Study Number and Site	Initial Dose, Schedule and Recommended Phase 2 Dose*	Modified Dose and Schedule
T98-0075 NCI-Navy	60 min IV q day X 5 days every 21 days (RPTD 40 mg/m^2)	60 min IV Days 1, 4, 15, 18 every 4 weeks starting dose 40 mg/m^2
T99-0035 MSKCC	60 min IV q day X 5 days every 21 days (RPTD 40 mg/m^2)	60 min IV Days 1, 4, 8, 15 every 21 days
T99-0058 Mayo	60 min IV Day 1, 8, & 15 every 28 days (RPTD 308 mg/m^2)	60 min IV Days 1, 4, 8, 11 every 21 days starting dose 57 mg/m^2
T99-0038 U Pitt	60 min IV Day 1, 8, & 15 every 28 days (RPTD 295 mg/m^2)	60 min IV twice weekly 3 of 4 weeks
T99-0013 Cancer Research UK	15-30 min IV weekly for 4 weeks (RPTD not established yet, Dose level 450 mg/m^2)	No change

* RPTD

important to note parenthetically that not all assessments of geldanamycin congener action in combination with conventional chemotherapeutic agents have indicated promise[13].

Finally, a pressing question remains whether additional geldanamycin analogs might be defined that have more desirable formulation or pharmaceutical advantages. This is particularly relevant as the DMSO/egg phospholipid-based vehicle that is necessary for 17-AAG is clumsy to apply to higher (>300 mg/M^2) doses. Other formulations of 17-AAG or congeners that are more soluble and orally bioavailable are being explored. For example, 17-dimethylaminoethylamino, 17-demethoxygeldanamycin (17-DMAG) has recently been defined as a water-soluble analog of 17-AAG that has comparable evidence of *in vivo* activity[14].

CONCLUDING REMARKS

Benzoquinoid ansamycins represent an as yet unexploited opportunity in cancer therapeutics. By interaction with the Hsp90 chaperone system, a substantial body of pre-clinical data would suggest that client proteins that are biologically important to the molecular economy of tumors, including oncogenes such as raf [20], erbB2 [21], cyclin D1 [22], met [23], Akt [24], steroid hormone receptors [12, 13], as well as other signaling systems [discussed in 24] can be perturbed. Therefore clinical investigations that assess these endpoints in tumor tissue will potentially be of great importance in clarifying the utility of approaching these molecular targets as a basis for useful advances in cancer chemotherapy. 17-AAG represents an initial example of agents that will address these endpoints, and it is hoped that the lessons learned from the development of 17-AAG or similar molecules will have general relevance to the evolution of other molecularly targeted agents. Remarkably, initial clinical experiences with 17-AAG have offered preliminary evidence that concentrations of the drug associated with activity in pre-clinical systems can be achieved in humans with tolerable toxicity, and provided early evidence of target modulation in at least certain surrogate and tumor compartments. Further studies with 17-AAG as well as potentially more pharmaceutically tractable analogs will be needed to assess the ultimate value of this approach to patients with cancer.

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