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Rapamycin with Antiretroviral Therapy in AIDS-Associated Kaposi Sarcoma: An AIDS Malignancy Consortium Study

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Abstract

Purpose—The mammalian target of rapamycin (mTOR) is activated in Kaposi sarcoma (KS) and its inhibitor, rapamycin, has induced KS regression in transplant-associated KS. This study aimed to evaluate rapamycin's safety and toxicity in HIV-infected individuals with KS receiving antiretroviral therapy (ART), investigate rapamycin interactions with both protease inhibitor (PI)-containing and non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing ART regimens, and assess clinical and biological endpoints including KS response and mTOR-dependent signaling.

Methods—Seven participants, 4 on PI-based and 3 on NNRTI-based ART, had rapamycin titrated to achieve trough concentrations of 5-10 ng/mL. Patients were monitored for safety and KS response. KS biopsies were evaluated for changes in phospho-Ribosomal S6 protein (pRPS6), and phospho-Akt expression. Interleukin-6 and vascular endothelial growth factor levels, HIV and KS-associated herpesvirus viral loads, and CD4 counts were monitored.

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Potential Conflicts of Interest:

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Results—Despite pharmacokinetic interactions resulting in >200-fold differences in cumulative weekly rapamycin doses between participants on PI-containing and NNRTI-containing regimens, treatment was well tolerated. There were no significant changes in viral loads or cytokine levels; modest initial decreases in CD4 counts occurred in some patients. Three participants, all on PI-containing regimens and with higher rapamycin exposure, showed partial KS responses. Three of four subjects whose biopsies were studied at \geq day 50 showed decreased pRPS6 staining.

Conclusions—Rapamycin appears safe in HIV-infected individuals with KS and can, in some cases, induce tumor regression and affect its molecular targets. Significant pharmacokinetic interactions require careful titration to achieve target drug trough concentrations, but may be exploited to achieve therapeutic benefit.

Keywords

Kaposi sarcoma; AIDS; rapamycin; mTOR; pharmacokinetic interactions

Introduction

Recognition that the phosphatidylinositol 3-phosphate kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway is dysregulated in many tumor types has led to numerous clinical trials of rapamycin (sirolimus) derivatives in various cancers, and their regulatory approval in a few¹⁻⁴. Although human immunodeficiency virus (HIV) infection has long been associated with an increased risk of AIDS-defining cancers, and more recently with an increased risk of certain non-AIDS-defining cancers (NADCs)⁵, the clinical use of rapamycin in HIV-infected individuals has focused on its immunosuppressive properties, chiefly to prevent rejection of solid organ allografts⁶⁻⁸ and HIV-infected patients were not included in pivotal trials leading to approval of rapamycin analogs for NADC indications.

Kaposi sarcoma (KS), the most commonly diagnosed AIDS-defining cancer worldwide, is one of several immunodeficiency-related neoplasms associated with infection with the KS-associated herpesvirus (KSHV)⁹. One mechanism by which KSHV may promote tumorigenesis is via activation of the phosphatidylinositol 3-phosphate kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) signaling pathway, which is dysregulated in many tumors (reviewed in¹⁰). In KSHV-associated tumors, several viral proteins can activate the PI3K/Akt/mTOR pathway in the absence of host mutations or deletions¹¹⁻¹⁴. The mTOR executes essential functions with respect to tumor cell growth and proliferation that result from Akt activation. Its potential as a target for KS therapy was suggested by the observation that KS regressed in a group of HIV-uninfected renal allograft recipients whose immunosuppressive therapy was changed from a cyclosporin A-based regimen to rapamycin, titrated to achieve trough concentrations of 6-10ng/ml, while their allograft function was not adversely affected¹⁵. In these patients, levels of phosphorylated Akt and p70S6 Kinase (a downstream target of the mTOR:Raptor complex, mTORC1, which phosphorylates ribosomal S6 protein, RPS6, to enhance translation) were elevated in pre-rapamycin KS biopsies compared to normal skin from the same individuals. These observations, supported by mechanistic studies showing direct inhibitory effects of rapamycin on KSHV-infected tumor cells^{11,13,16} and indirect, immunomodulatory effects leading to enhanced recovery of T-cell responses to KSHV¹⁷, provided a rationale for evaluating rapamycin in HIV-associated KS.

Because rapamycin is a substrate for the drug-metabolizing enzyme cytochrome P450 3A4 (CYP3A4) and the efflux transporter P-glycoprotein^{18,19}, we anticipated interactions with antiretroviral drugs (ART) that inhibit or induce these proteins. Indeed, when this study was

initially developed in 2006, we knew of 2 reports in HIV-infected transplant recipients showing interactions between drugs used to prevent graft rejection and ART^{6,7}. However, these reports provided insufficient data on which to base firm recommendations about appropriate rapamycin doses to use together with different ART regimens. We also had concerns about administering an immunosuppressive agent to individuals infected with an immunosuppressive virus, but this was tempered by reports that rapamycin inhibits HIV infectivity and transcription in culture²⁰.

With the foregoing in mind, the AIDS Malignancy Consortium (AMC), a National Cancer Institute-supported clinical trials group, designed a pilot study, AMC051, to evaluate rapamycin's safety and toxicity in HIV-infected individuals with KS receiving protease inhibitor (PI)-based or non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral regimens and to estimate the dose(s) of rapamycin required to achieve trough rapamycin concentrations between 5 and 10 ng/mL. These aims are consistent with the goals, articulated by Persad et al²¹ and endorsed by the AMC, of identifying significant interactions between new anticancer agents and drugs used to treat HIV infection and removing barriers to enrollment of HIV-infected cancer patients into clinical trials. As secondary objectives, we wished to evaluate the clinical response of KS to rapamycin and its effects on mTOR-dependent signaling, serum cytokines, HIV and KSHV viral loads, and CD4 T-lymphocyte counts.

Patients and Methods

Patients

Eligible participants were HIV-infected men or women, ≥ 18 years old, with stable or progressing, biopsy-proven KS, who were receiving a stable anti-retroviral regimen of at least three drugs, one of which had to be a PI or an NNRTI, for ≥ 12 weeks. At least five measurable, non-radiated, cutaneous indicator lesions, as well as additional skin lesions for biopsy, were required. Lymph node, oral, gastrointestinal (GI) and/or lung KS, not requiring cytotoxic therapy, was permitted. Additional requirements included Karnofsky performance status (KPS) > 60 , life expectancy ≥ 3 months, and ability to provide informed consent and comply with the protocol. Effective barrier contraception was required of all participants; women of child-bearing potential were required to have a negative pregnancy test within 72 hours. The protocol and consent form were approved by each of the participating sites' institutional review boards in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services.

The following laboratory parameters were required within 21 days before entry: hemoglobin ≥ 8.0 gm/dL; neutrophils ≥ 1000 cells/mm³; platelets $\geq 75,000$ /mm³; GFR > 40 mL/min; total bilirubin ≤ 1.5 X the upper limit of normal (ULN), with exceptions for elevated indirect bilirubin in subjects receiving indinavir or atazanavir; AST and ALT ≤ 2.5 times ULN; fasting triglycerides ≤ 400 mg/dL (4.5 mmol/L), and total cholesterol ≤ 300 mg/dL (7.8 mmol/L); spot urine protein:creatinine ratio ≤ 0.5 and/or proteinuria ≤ 500 mg/day; and, CD4 count > 50 cells/ μ L and plasma HIV RNA level < 400 copies/mL.

Exclusion criteria included prior rapamycin treatment; active infection; prior or concurrent malignancy except basal cell skin cancer or cervical carcinoma *in situ*; treatment for infection or other serious illness within 14 days; infiltrate, cavitation or consolidation on chest x-ray within 3 months; treatment for KS within four weeks or local therapy to any KS indicator lesion within 60 days; investigational treatments within four weeks; acute or chronic liver disease; and/or, Grade III/IV cardiac disease. Nursing or pregnant women were excluded. Systemic corticosteroids and agents other than ART that would interfere with rapamycin metabolism or excretion were prohibited.

Treatment

The protocol originally specified an initial rapamycin dose of 0.025 mg/kg/day using a liquid oral formulation (Rapamune®, Wyeth Pharmaceuticals, Inc, Philadelphia, PA) at a concentration of 1 mg/mL, without a loading dose. We planned to treat 6 evaluable participants, 3 each on PI-containing and NNRTI-containing antiretroviral regimens. After the first study participant treated at this dose together with a ritonavir-boosted PI (PI/r) regimen showed an unacceptably high trough concentration after 7 days, we modified the starting doses based on analysis of data from the first patient and the literature as follows: participants receiving a PI/r regimen received 0.0015 mg/kg/day; those receiving a PI regimen without ritonavir received 0.003 mg/kg/day; and, those on an NNRTI regimen received 0.05 mg/kg/day. If the calculated daily dosage yielded volumes ≤ 0.3 mL, the daily value was multiplied by 2 or 3 to yield a volume of at least 0.3 mL; the corresponding doses were administered three times weekly or twice weekly, respectively. Dosing was subsequently adjusted based on trough blood rapamycin concentrations to achieve target concentrations between 5 and 10 ng/mL. Responding patients could be treated for up to 12, four-week cycles. Participants with progressive KS at any time, or those without objective response after 6 cycles, were removed from study.

Monitoring

Routine clinical and laboratory assessments were performed at baseline, days 8, 15 and 29, and every 4 weeks thereafter. KS evaluations were performed as described previously²² at baseline; response was assessed every 4 weeks for the first 3 months, and every 8 weeks thereafter. Patients completing treatment with stable disease or objective response had their response status reassessed approximately 30 days after the last rapamycin dose. CD4+ T-lymphocyte count and HIV VL were measured at baseline, week 4, and then every third cycle. KSHV VL and cytokines were measured at baseline and Day 15 of cycle 1 and on Day 1 of cycles 2, 3, 5, 7, 9, and 11. Rapamycin trough concentrations were measured on Days 8, 15, 21, 29, 43 and 57 and every 4 weeks thereafter. We initially monitored trough concentrations by liquid chromatography tandem mass spectroscopy (LCMSMS) (Quest Diagnostics, San Juan Capistrano, CA). However, because results using this method were not available until ≥ 3 days after the specimen was obtained, we added backup rapamycin immunoassays to facilitate recognition of potentially toxic levels. Immunoassays were performed on an IMx analyser (Abbott Laboratories, Chicago, IL, USA) by microparticle enzyme immunoassay (MEIA), with results available the same day. Once a participant's dose stabilized and LCMSMS trough values were consistently within the target range, backup immunoassays were discontinued. Rapamycin doses were adjusted when trough concentrations fell outside the target range as follows: new dose = current dose \times (midrange target trough concentration/actual trough concentration), where the midrange target is 7.5 ng/mL.

KS punch biopsies were performed at baseline and again within 14 days of achieving a trough rapamycin concentration within the target range. Specimens were fixed in 10% formalin.

KSHV viral load (VL)

Plasma was separated from whole blood using Ficoll-based gradient centrifugation and 100 μ L was used to obtain genomic DNA using the Abbott m2000 DNA Sample Preparation System (Abbott Laboratories) according to the manufacturer's protocol. Quantitative real-time polymerase chain reaction was performed as described²³.

HIV VL was measured using standard commercial assays having a lower limit of sensitivity of 50 RNA copies/mL.

Cytokines

Human interleukin-6 (IL6) and vascular endothelial growth factor (VEGF) were measured in plasma using enzyme-linked immunosorbent assay kits from eBiosciences (San Diego, CA) and PeproTech (Rocky Hill, NJ), respectively.

Immunohistochemistry (IHC)

Formalin-fixed, paraffin-embedded tumor biopsies were cut into 7 μ m sections, de-paraffinized, rehydrated, and incubated in 3% hydrogen peroxide/10% methanol to block endogenous peroxidase. Samples were boiled for 20min in 1mM EDTA (pH 8.0) and blocked with 10% horse serum (Vector Laboratories, Burlingame, CA) in PBS/5% BSA/0.3% TritonX-100 (Blocking Buffer), then incubated with primary antibody diluted 1:100 in Blocking Buffer overnight at 4°C. The antibodies used were: phospho-RPS6 (pRPS6), phospho-Akt (Ser473) and phospho-Akt (T308) (Cell Signaling, MA). Primary antibodies were detected with VectaStain ABC kit (Vector Labs, Burlingame, CA) and NovaRed™. Sections were counterstained with hematoxylin, mounted, and imaged using a LEICA DM microscope (Leica GmBH, Heidelberg, Germany) equipped with a 10/0.25 numerical aperture (NA) or a 40/0.75 NA N plan objective and Leica DPC480 camera.

KSHV latency associated nuclear antigen (LANA) and Ki-67 stains were performed using the BOND-MAX Autostainer (Leica Microsystems, Bonnockburn, IL) using the accompanying Bond polymer define detection kit after antigen retrieval with Bond epitope retrieval solution 2 (Leica Microsystems). The antibodies used were KSHV LANA (clone LN3; Advanced Biotechnologies, Inc., Columbia, MD) and Ki-67 (MIB-1) (DakoCytomation, Carpinteria, CA).

Biopsies were analyzed by visual scanning of the entire section. Samples were scored blinded on a scale of 0 to 4 where 0 indicates no staining and 4 is staining equivalent to the positive control. No staining (red color) observed in the absence of primary antibody was used as a negative control. Changes in staining score and KSHV VL from baseline to post-treatment were evaluated using the Wilcoxon signed rank test.

Results

Patient Characteristics

Characteristics of the seven enrolled participants are shown in **Table 1**. They uniformly showed high CD4 T-lymphocyte counts and near-normal KPS. The diagnosis of KS was confirmed by positive LANA staining in all baseline biopsy specimens (data not shown).

Rapamycin Dose Titration

Table 2 summarizes rapamycin doses and trough levels. All four participants who received PI/r-containing ART initially overshot the maximum target trough concentration of 10ng/ml and required multiple adjustments to regulate the dose. Indeed, the first patient, who was receiving a ritonavir-boosted lopinavir regimen, showed a trough level of 123ng/mL after 7 days of treatment at a rapamycin dose of 2mg once a day. The commercial laboratory performing the drug assays assumed that the level was an error and refused to release the data to the treatment site until the assay had been repeated, delaying dosage interruption for another week, at which time the blood level had reached 172ng/mL. After stopping rapamycin, blood levels gradually declined to acceptable levels over three weeks. Despite these high levels, he showed minimal (grade 1) side effects. Although two subjects on NNRTI-based regimens initially overshot the maximum target trough level, only single dosage adjustments were needed to achieve consistent trough levels within the target range; one subject on an NNRTI-based regimen required an increase in the initial dose. As shown

in Table 2, the eventual stable maintenance doses for the four individuals on PI/r-containing ART regimens ranged from 0.1mg twice weekly to 0.3mg three times a week, whereas for participants on NNRTI-based regimens, the range was 2.3 to 6.7mg once a day, a difference of >200 fold.

Adverse Events

Treatment was generally well tolerated. All patients experienced adverse events, but most were grade 1. Two patients had serious adverse events: grade 3 infection (dental abscess) without neutropenia, attributed to a pre-existing condition (root canal) in patient #1 (this event occurred several months after the rapamycin trough level had fallen to within the desired target range), and extensive grade 3 superficial vein thrombus requiring anticoagulation, considered possibly related to rapamycin, in patient #4.

Patient #5 had 2 separate grade 2 infectious episodes without neutropenia, including pneumonia responsive to oral antibiotics and dermatomal Herpes zoster. Patient #6 developed symptoms of a grade 2 upper respiratory infection, without neutropenia, that resolved without treatment. Four patients (two each on PI- and NNRTI-based regimens) experienced triglyceride elevations, with a maximum grade of 1 (1 patient), 2 (2 patients) and 3 (1 patient).

Effects on markers of HIV Disease

We observed no consistent changes in HIV VL. Four of five participants with undetectable HIV VLs at baseline showed no change during therapy, whereas one patient showed an isolated increase at week 40 and again had an undetectable level at week 48. One participant showed a baseline value just above the limit of assay detection (65 copies/mL), but was not subsequently tested. Another participant with an undetectable VL at screening was later discovered to have become non-adherent to antiretroviral treatment in the interval between screening and initiation of rapamycin, and had an elevated HIV VL at day 1. He subsequently resumed antiretroviral therapy and his VL remained undetectable from week 16 through week 48.

The median baseline CD4 count was 826 cells/ μ L (range, 558-1062). CD4 counts remained >400 cells/ μ L for the 6 study participants in whom follow-up tests were performed (**FIGURE 1**). Five of these 6 patients showed a >10% drop in CD4 count within the first 16 weeks. Afterwards, CD4 counts increased to baseline levels or remained level.

Effects on KSHV plasma VL

At baseline, the median KSHV VL was 214 copies/ml (range, 28-56,653). There were no significant changes during rapamycin treatment. The median change during treatment was -138.8 copies/ml (P=0.156).

Therapeutic response (Table 2)

The median treatment duration was 16 weeks (range, 4-49 weeks). Three patients showed partial KS response after 3 to 9 weeks. Response duration ranged from 43 to 50 weeks. None of the responding patients relapsed during study treatment or at the 30-day follow-up. The three patients showing partial response were all receiving PI/r regimens, and were also the patients showing the highest rapamycin trough levels. Three patients showed stable disease as the best response and one showed KS progression after 4 weeks. Two stable patients subsequently showed KS progression at weeks 12 and 17 of study, respectively; the third patient discontinued study participation at week 14 (relocated to another state) without having shown KS progression.

Plasma cytokine concentrations

At baseline, the median plasma IL6 concentration was 8.22pg/ml (range, 3.00-23.5) and the median plasma VEGF concentration was 187ng/ml (range, 40.4-406). No significant changes in plasma IL6 or VEGF concentrations were observed during treatment (data not shown).

KS biopsy studies

Figure 2 shows IHC for pRPS6 in KS lesions at baseline. The top right panel shows a biopsy from subject #6, in which pRPS6 was detected both in KS spindle cells and endothelial cells lining blood vessels. The other panels show the variation in pRPS6 positivity in baseline biopsies.

All patients had at least one biopsy repeated while receiving rapamycin. Four patients, including the three who received NNRTI-based regimens and one (subject #3) who received a PI/r-based regimen, had biopsies repeated between days 22 and 29. All four patients who received PI/r-based regimens had biopsies performed at day 50 or later. On-treatment biopsies were assessed for changes from baseline in pRPS6, phospho-Akt (Ser 473) and phospho-Akt (T308) staining. Although overall, no significant changes were detected in staining scores, the biopsies of some individuals showed evidence for pRPS6 inhibition. **Figure 3A** shows a representative section stained for pRPS6 at baseline, and **Figure 3B** a section from the same patient after 50 days on drug. In this individual, decreased pRPS6 reactivity was observed within KS tumor cells and also within overlying epithelium. As shown in **Figure 3C**, three of four subjects whose biopsies were studied at day 50 or later – all of whom received PI/r-based ART regimens – showed decreased pRPS6 staining compared to baseline. In all four, the on-study staining level was absent (score 0) or minimal (score 1), suggesting that rapamycin had affected its molecular target.

We saw no significant changes in Akt phosphorylation at either T308 or Ser473, or changes in Ki-67 or LANA staining upon treatment with rapamycin (data not shown).

Discussion

KS was one of the first clinical manifestations heralding the acquired immunodeficiency syndrome. Although KS incidence in the U.S. declined after peaking in the 1980s, it has recently stabilized^{24,25}. At the same time, improved ART has prolonged the lives of many HIV-infected individuals, resulting in increased numbers of people living with HIV and at risk for various cancers, including KS. In low-resource settings, particularly sub-Saharan Africa where rates of both HIV and KSHV infection are much higher than in the U.S. and antiretroviral therapy has reached only a fraction of affected individuals, KS remains common²⁶⁻²⁹. In both high- and low-resource settings, improved KS therapy is needed.

Rapamycin analogs have received U.S. Food and Drug Administration approval for treatment of renal cell carcinoma, and other tumors have been shown to be sensitive to these agents^{1,3}. These tumors are considered PI3K/Akt/mTOR addicted. They are characterized molecularly by high-level phosphorylation of Akt, mTOR and the mTORC1 targets, p70S6 Kinase and RPS6. KS lesions also express phosphorylated Akt^{15,30} and as shown here in Figure 1, phosphorylated RPS6. Therefore, KS can be considered a PI3K/Akt/mTOR-addicted tumor.

Our findings are consistent with those in HIV-negative renal allograft recipients¹⁵, and demonstrate that rapamycin, an allosteric inhibitor of mTORC1, can induce KS regression in at least some HIV-infected individuals without inducing serious adverse effects. However, the small sample makes any statement about the efficacy of rapamycin in AIDS-associated

KS premature. Treatment was, overall, well tolerated even when initial rapamycin trough levels markedly overshoot the intended range. Notably, there was no increase in HIV VL, which was not unexpected given that HIV replication depends on activated T cells and active Akt signaling^{20,31}. We did, however, observe modest decreases in CD4 T-lymphocyte counts in some participants. Because they all had relatively high baseline CD4 T-lymphocyte counts, the decrease did not result in levels that would raise concern about susceptibility to opportunistic infections typically associated with advanced HIV disease. Although three patients experienced infections, they were self-limited and/or easily treatable, were not AIDS-defining, and may not have been related to rapamycin administration. A CD4 count decrease of similar magnitude among patients starting at a lower baseline level could, however, be of concern.

We observed substantial interactions between rapamycin and antiretroviral drugs, most notably PI/r. Although interactions were expected based on the prior experience in HIV-infected transplant recipients^{6,7} their magnitude and the speed with which elevated levels developed was not, and required marked reduction of the originally planned rapamycin dose in subjects receiving ritonavir, a particularly potent inhibitor of CYP3A4³²⁻³⁷. While it was possible to suggest a starting dose in these patients, therapeutic monitoring and dose readjustments were necessary because of the large inter-patient variability in rapamycin pharmacokinetics and the variable effect of ART on its pharmacokinetics. These types of interactions have implications for the management of the growing number of HIV-infected cancer patients with the many anticancer agents whose absorption and/or metabolism are regulated by CYP enzymes.

The observation that KS regression was documented only in subjects receiving PI/r-based regimens deserves comment. Given the small sample, this may have been a chance occurrence. It is possible, however, that increased and/or more sustained rapamycin exposure accounts for both the improved therapeutic effects and the more consistent decreases in pRPS6 staining in biopsies from those patients. Alternatively, or in addition, some of the “off-target” effects of PIs, which include inhibition of Akt activation (reviewed in ³⁸), may have contributed to the observed therapeutic effects. These possibilities require further study. Finally, we note that dose-related side effects for rapamycin are not well defined. Among our patients who inadvertently achieved very high rapamycin levels, it is not clear that there were resultant adverse effects. A future study with dose escalation to levels of rapamycin higher than those targeted for chronic immunosuppression in the context of organ transplantation may be warranted. This study suggests that in patients on PI/r regimens, much higher, sustained drug levels could be readily achieved.

In conclusion, the results of this study suggest that rapamycin, and potentially other “rapalogs”, can be safely administered to patients with HIV-infection and cancer. Rapamycin induced both regression of HIV-associated KS and predicted effects on mTOR targets in tumor biopsies in some patients. Thus we believe that studies in a larger group of patients and perhaps involving dose escalation are warranted.

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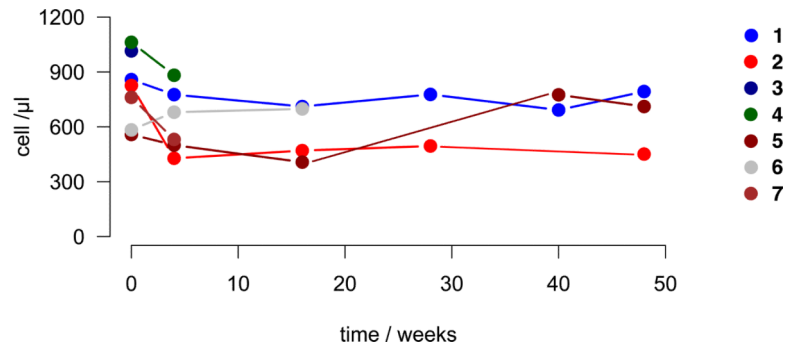


Figure 1. Changes in CD4 T-lymphocyte counts during rapamycin treatment. Shown are patient CD4 counts in cells per μl on the vertical and time on protocol in days on the horizontal axis. The different colors represent individual patients.

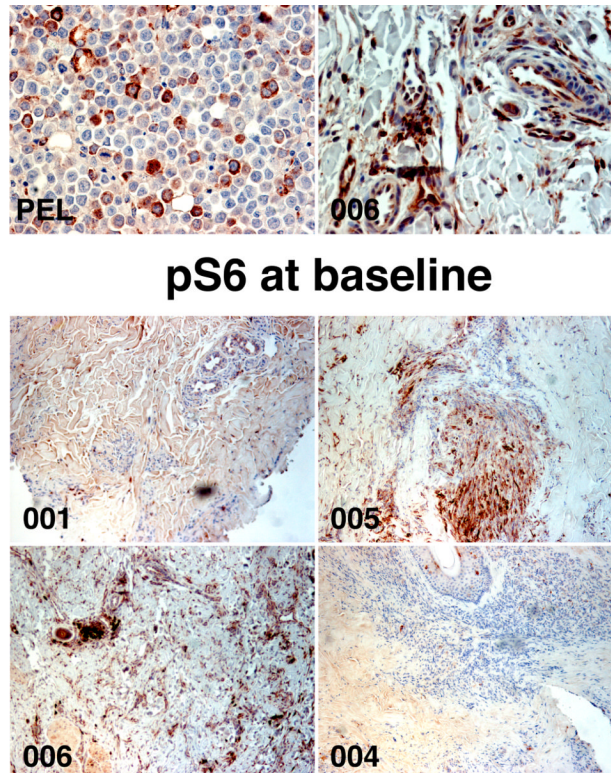


Figure 2. Phospho RPS6 (S235/S236) staining at baseline. Shown are representative biopsies from 4 patients prior to treatment. A xenograft tumor that developed after subcutaneous injection of primary effusion lymphoma (PEL) BC-1 cells was used as a positive control (top left panel). The red precipitate indicates the presence of phosphorylated RPS6 in a cell. Cells were counterstained with hematoxylin (blue). Top two panels, 400x magnification, bottom four panels at 100x magnification.

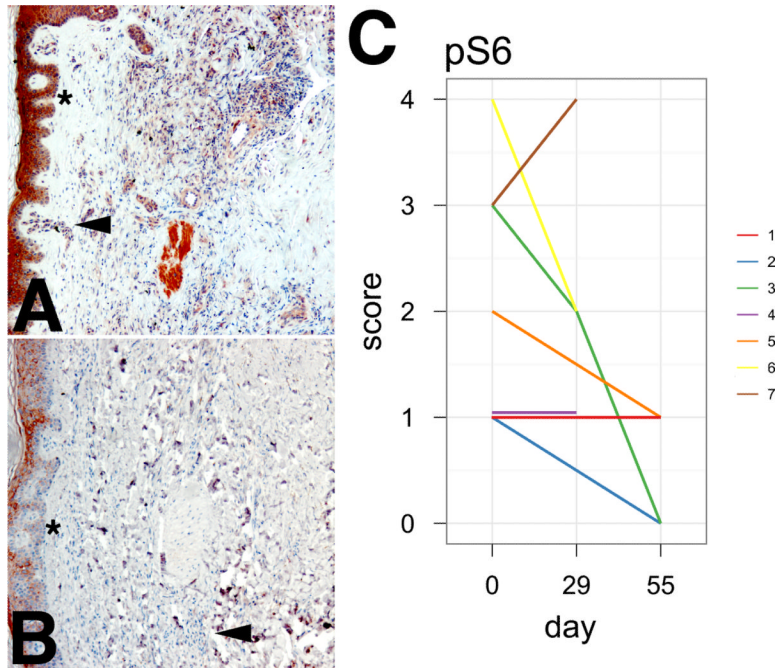


Figure 3. Phospho RPS6 (S235/S236) staining before and after exposure to rapamycin. Panels A and B show representative biopsies at baseline and after 50 days of treatment in subject #5, respectively. Panel C shows a quantitation of the IHC data for all patients. The intensity score is shown on the vertical axis and time in days on the horizontal axis. Individual patients are indicated by different colors. Note that most patients had only two biopsies (baseline and after either 22-29 or ≥ 50 days) as specified in the study protocol.

Table 1

Selected Baseline Characteristics

	N (%)
Male Gender	7 (100)
Non-Hispanic, White	5 (71)
Non-Hispanic, Black	2 (29)
Age (Years)	
N	7
Mean \pm SD	46.3 \pm 6.2
Median	44.0
Range	39 -55
Absolute CD4 Count (cells/μL)	
N	7
Mean \pm SD	809.0 \pm 193.9
Median	826.0
Range	558 - 1062
HIV Viral Load <50 copies/mL	5 (71)
Extent of KS	
>50 skin lesions	5 (71)
Tumor-associated edema	6 (86)
Oral or visceral	0 (0)
HAART Regimen	
Ritonavir-boosted PI-based	4 (57)
NNRTI-based	3 (43)
Prior KS Treatment	5 (71)
Liposomal doxorubicin	4 (57)
Paclitaxel	2 (29)
Bleomycin/vincristine	1 (14)
Doxorubicin/bleomycin/vincristine	1 (14)
Interferon alfa	1 (14)
IM 862	1 (14)
Valproic acid	1 (14)
Halofuginone	1 (14)
COL-3	1 (14)
Imatinib	2 (29)

Table 2

Rapamycin Doses, Maximum Trough Levels and KS Response

Patient Number	Baseline Weight (kg)	PI or NNRTI Received	Initial Rapamycin Regimen	Maintenance Rapamycin Regimen at Target Trough	Maximum Rapamycin Trough Level (ng/mL) by LCMSMS	Best Response of KS to Rapamycin
001	78.5	Lopinavir/r	2 mg daily	0.2 mg biw [*]	172	Partial
002	64.9	Atazanavir/r	0.3 mg biw	0.2 mg biw	64.4	Partial
003	74.5	Atazanavir/r	0.3 mg biw	0.3 mg tiw	16.8	Stable
005	83.9	Lopinavir/r	0.4 mg biw	0.1 mg biw	19.2	Partial
004	97.0	Nevirapine	4.9 mg daily	2.8 mg daily	13.1	Progression
006	90.3	Efavirenz	4.5 mg daily	6.7 mg daily	7.4	Stable
007	71.6	Efavirenz	3.7 mg daily	2.3 mg daily	11.9	Stable

* Abbreviations: biw, twice a week; tiw, three times a week; /r, ritonavir-boosted protease inhibitor