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Weekly *nab*-Rapamycin in Patients with Advanced Nonhematologic Malignancies: Final Results of a Phase 1 Trial

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Abstract

Purpose—This dose-finding phase 1 study investigated the maximum tolerated dose (MTD) and safety of weekly *nab*-rapamycin in patients with untreatable advanced nonhematologic malignancies.

Experimental Design—*nab*-Rapamycin was administered weekly for 3 weeks followed by 1 week of rest, with a starting dose of 45mg/m². Additional doses were 56.25, 100, 150, and 125 mg/m².

Results—Of 27 enrolled patients, 26 were treated. Two dose-limiting toxicities (DLTs) occurred at 150mg/m² (grade 3 AST elevation and grade 4 thrombocytopenia), and 2 DLTs occurred at 125mg/m² (grade 3 suicidal ideation and grade 3 hypophosphatemia). Thus, the MTD was declared at 100mg/m². Most treatment-related adverse events (TRAEs) were grade 1/2, including thrombocytopenia (58%), hypokalemia (23%), mucositis (38%), fatigue (27%), rash (23%), diarrhea (23%), nausea (19%), anemia (19%), hypophosphatemia (19%), neutropenia (15%), and hypertriglyceridemia (15%). Only 1 grade 3 nonhematologic TRAE (dyspnea) and 1 grade 3 hematologic event (anemia) occurred at the MTD. One patient with kidney cancer had a partial response. The longest clinical benefits were observed in 2 patients: 1 patient with mesothelioma (SD: 365 days) and 1 patient with neuroendocrine tumor (SD: 238 days). The C_{max} and AUC

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Conflict of Interest Statement

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increased with dose between 45–150mg/m², except for a relatively low AUC at 125 mg/m². *nab*-Rapamycin significantly inhibited mTOR targets S6K and 4EBP1.

Conclusions—The clinical dose of single agent *nab*-rapamycin was established at 100 mg/m², which was well tolerated with preliminary evidence of response and SD, and produced a fairly dose proportional pharmacokinetic profile in patients with unresectable advanced nonhematologic malignancies.

INTRODUCTION

The prognosis for patients with advanced solid tumors is poor, as most malignancies are not responsive to standard treatments at the advanced stage. mTOR, a serine/threonine-specific protein kinase, is downstream of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, and a key regulator of cell survival, proliferation, stress, and metabolism (4). mTOR inhibition with rapamycin and rapalogs (everolimus, temsirolimus) has proven to be effective in various solid tumors including renal cell carcinoma, neuroendocrine tumors, and breast cancer (5–15).

While rapamycin is an efficacious allosteric inhibitor of mTOR complex 1 (mTORC1), it has low oral bioavailability, poor solubility, and dose-limiting intestinal toxicity (16, 17). Other rapalogs, including everolimus and ridaforolimus, are also oral preparations and are often associated with significant stomatitis (1). Temsirolimus, a prodrug of rapamycin, requires conversion by the CYP3A enzyme and also carries a significant risk for developing skin rash and stomatitis (18). Because none of the rapalogs are highly water soluble, they require surfactants and solvents in an intravenous formulation, such as polysorbate 80 for temsirolimus (19). The use of surfactants can potentially cause irritation, local inflammation, and potential reduction of drug efficacy due to micellar sequestration, and the need for premedication to avoid potential hypersensitivity reactions (19). The nanoparticle albumin-bound rapamycin (*nab*-rapamycin, Celgene Inc, Summit, NJ), with a mean particle size of about 100 nm, is freely dispersible in saline and is suitable for intravenous administration, and may be an advantageous alternative to oral rapamycin or oral rapalogs. Human albumin has broad binding affinity and accumulates in tumors, making it an ideal candidate for drug delivery (20, 21). In accordance with these observations, *nab*-paclitaxel, the first therapeutic agent developed by the *nab*TM technology (Celgene), showed improved drug delivery, safety, and efficacy compared with the conventional solvent-based paclitaxel in various solid tumors (22, 23). In preclinical studies, *nab*-rapamycin has shown an excellent efficacy and safety profile; it reduced cell viability and decreased downstream signaling in various xenograft cancer models, including pancreatic, colorectal, multiple myeloma, and breast cancer (24–27). Additionally, in human breast xenograft models, *nab*-rapamycin alone produced 75% tumor growth inhibition without weight loss and antitumor activity was further enhanced with the combination of doxorubicin (a topoisomerase inhibitor), SAHA (an HDAC inhibitor), erlotinib (an epithelial growth factor tyrosine kinase inhibitor), and perifosine (an Akt inhibitor) with 15% weight loss, indicating high tolerability in the combination regimens (28).

Based on the promising preclinical results, this dose-finding phase 1 study investigated the maximum tolerated dose (MTD) and safety of intravenous single agent weekly *nab*-rapamycin in patients with untreatable advanced nonhematologic malignancies.

PATIENTS AND METHODS

This study was conducted at MD Anderson Cancer Center, TX, and the Sarcoma Oncology Center, Santa Monica, CA. The study was approved by the Institutional Review Board of both participating medical institutions and was conducted in compliance with the World

Medical Association Declaration of Helsinki and Good Clinical Practice, Guidelines of the International Conference on Harmonization (29). Written informed consent was obtained from all patients before study initiation.

Patients

Eligible patients were 18 years old, had histologically or cytologically confirmed diagnosis of stage IV cancer which was not amenable to curative therapy. Advanced disease was defined as metastatic disease or locally advanced disease that was surgically unresectable and considered unmanageable with standard therapies such as radiation or systemic therapies. Patients had a measurable disease by Response Evaluation Criteria in Solid Tumor (RECIST) v1.0, life expectancy ≥ 3 months, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, adequate renal function (serum creatinine <1.5 mg/dL and/or creatinine clearance ≥ 60 mL/min), and were off all therapy for at least 4 weeks prior to study drug administration. Patients were excluded from the study if they had brain metastasis, history of interstitial lung disease and/or pneumonitis, or a history of allergy or hypersensitivity to the study drug or any compounds of similar chemical or biological composition.

Study Design

This dose-finding study evaluated MTD and dose limiting toxicities (DLTs) of *nab*-rapamycin in patients with advanced nonhematologic malignancies. Following baseline evaluations, patients entered into the treatment period. *nab*-Rapamycin was administered by intravenous infusion for 30 minutes weekly for 3 weeks followed by 1 week of rest (28-day cycle), with a starting dose of 45 mg/m². The starting dose of *nab*-rapamycin was chosen based upon nonclinical toxicology data of *nab*-rapamycin. Additional dose levels were 56.25, 100, 150, and 125 mg/m². The original protocol was amended to add the 125 mg/m² dose cohort for refinement of MTD.

The first cycle was considered the treatment interval for determination of DLTs and the MTD. The MTD for *nab*-rapamycin was determined using a standard 3+3 design, where 3 patients were enrolled at each dose level. If no DLT was observed, 3 additional patients were enrolled at the next dose level. If 1 DLT was observed, the dose level was expanded to 6 patients. If 2 DLTs were observed at a given dose level, the MTD was considered to be exceeded. Of the 6-patient expanded cohort, if 1 out of 6 patients experienced a DLT, this was defined as the MTD. All patients at a given dose level completed 1 cycle of therapy before patients were enrolled at the next dose level.

A DLT was defined (using the National Cancer Institute Common Terminology Criteria of Adverse Events [NCI CTCAE] v3.0 as any grade 3/4 nonhematologic toxicity, grade 3/4 nausea or vomiting that occurred despite treatment, grade 4 thrombocytopenia of any duration and grade 4 uncomplicated neutropenia (ie, without fever or infection) lasting >7 days, grade 4 febrile neutropenia that required hospitalization, and any grade 3 hematologic toxicity that required treatment delay beyond 3 weeks.

Throughout the study, patients were routinely assessed for toxicities, response, and possible need for a dose modification. Patients continued on treatment until they experienced progressive disease (PD) or unacceptable toxicity, withdrew consent, or their physician felt it was no longer in their best interest to continue on treatment. Discontinued patients completed the end of study evaluation and entered into a 30-day follow-up period.

Assessments and Statistical Methods

All patients who received at least 1 dose of study drug (treated population) were evaluated for safety. Safety and tolerability endpoints included the incidence of treatment-related adverse events (TRAEs) by NCI CTCAE v3.0 and the percentage of patients experiencing TRAEs that required dose delays/modifications, and/or premature discontinuation of the study drug.

The efficacy endpoints included the percentage of patients who achieved an objective confirmed complete or partial tumor response (CR or PR, respectively) and the percentage of patients with confirmed stable disease (SD) for at least 12 weeks, using RECIST v1.0. The objective tumor responses of target or nontarget lesions were classified individually based on RECIST v1.0. The overall tumor response was determined by taking into account the responses of target lesions and nontarget lesions as well as the presence of new lesions.

Tumor response assessments were done every 12 weeks. A waterfall plot was used to illustrate the percentage change of target lesion from baseline for all patients with target tumor evaluation. The corresponding objective target lesion responses, dose level cohorts, and tumor types were also provided in the graph.

Molecular Analyses

Evaluation of phosphatase and tensin homolog (PTEN) loss was performed with immunohistochemistry (IHC) using monoclonal mouse anti-human PTEN antibody clone 6H2.1 from Dako at 1:100 dilution, as described in Gonzalez-Angulo, 2011 (30). Briefly, both cytoplasmic and nuclear PTEN staining in the tumor and non-neoplastic ductal epithelium and stroma were quantified. PTEN expression level was scored semiquantitatively on the basis of staining intensity (SI) and distribution using the immunoreactive score (IRS) as follows: $IRS = SI \times \text{percentage of positive cells (PP)}$. SI was determined as 0, negative; 1, weak; 2, moderate; and 3, strong. PP was defined as 0, <1%; 1, 1%–10%; 2, 11%–50%; 3, 51%–80%; and 4, >80% positive cells. Tumors with IRS of 0 were considered to have PTEN loss. A mass spectroscopy-based approach evaluating single nucleotide polymorphisms was used to detect known mutations in members of the PI3K pathway. Molecular analysis was performed in patients who demonstrated clinical benefit using archival tissue.

Pharmacokinetics

Whole blood samples (4 mL each) were collected in vacutainer tubes containing EDTA as the anticoagulant for determination of rapamycin. Samples were obtained only during cycle 1 and were taken immediately predose (before infusion), during the infusion (15 and 30 minutes before end of the infusion), and postinfusion at 1.0, 1.5, 2, 4, 6, 8, 24, 48, 72, 96, and 168 hours. The samples were stored frozen at a temperature between –20 and –80°C until shipment for analysis to St. George's Hospital at the University of London.

The whole-blood samples were analyzed for total (free + bound) rapamycin using high performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS). Rapamycin concentrations in whole blood were validated from 10 to 2000 ng/mL with 32-desmethoxyrapamycin used as an internal standard. Analytes were extracted using a solvent mixture and detected and quantified by reverse phase high-performance liquid chromatography with detection via turbo ion-spray mass spectrometry.

The concentration-versus-time data for rapamycin in whole blood were analyzed using a noncompartmental analysis technique and WinNonlin software. Pharmacokinetic analysis was based on whole blood concentrations due to the known instability of rapamycin in

plasma. Calculated parameters included peak concentration (C_{max}), half-life ($t_{1/2}$), area under the concentration-time curve (AUC), clearance (CL), and steady-state volume of distribution (V_{ss}). A simple regression model was applied to assess the relationship of the pharmacokinetic parameters with dose.

Peripheral Blood Mononuclear Cells (PBMCs) and Reverse Phase Protein Arrays (RPPA)

Whole blood for pharmacodynamics evaluation was collected only during cycle 1 at 4 time points: C1 D1 (pretreatment), C1 D2, C1 D4, and C1 D8 (immediately prior to next dose) in an 8 mL cell preparation tube with sodium citrate (Becton, Dickinson and Company, Franklin Lakes, NJ). Separation of PBMCs from whole blood was accomplished through density gradient centrifugation using Ficoll following manufacturer's recommendations. After centrifugation, plasma component from the upper half of the tube was transferred to cryotubes and snap-frozen. The layer containing the cells was transferred to a fresh tube, washed and centrifuged. After removal of the supernatant, PBMC pellet was also snap-frozen.

Reverse phase protein array was performed in the MD Anderson Cancer Center Functional Proteomics RPPA Core Facility as described previously (31). PBMC samples were resuspended in RPPA lysis buffer containing 0.25% sodium deoxycholate. Protein concentrations were determined using BCA method (Pierce, Rockford, IL) and 4x SDS sample buffer was added. Final protein concentration was adjusted to 3 $\mu\text{g}/\mu\text{L}$. Samples were probed with antibodies that were validated for RPPA. A total of 135 proteins and 21 replicates were analyzed, including S6 S240/244, S6 S235/236, S6KT389, 4EBP1 T37/46, and 4EBP1 T70. Proteomics assessment of S6 S240/244 and 4EBP1 T37/46 was carried out using Mesoscale discovery (MSD) phosphoprotein assays (Meso Scale Discovery, Gaithersburg, MD).

The RPPA spot signal intensity data obtained from MicroVigene automated RPPA module (VigeneTech, Inc., North Billerica, MA) was analyzed using the R package SuperCurve (version 1.4.3) (32), available at "<http://bioinformatics.mdanderson.org/OOMPA>". RPPA raw data were treated with median centering across samples; and then a centering by the sample median was undertaken on the treated data and the final normalized data were obtained by applying median absolute deviation (MAD) scaling to the data. Linear mixed models and ANOVA tests were developed and applied to test the pre- vs post-treatment and inhibition effects at each dose level and each pair of time points. Tukey's tests were also used for pairwise comparisons. In order to test the association of proteins expression on patients' response, SD and PD patients were also compared using logistic models adjusted by time points, and their interactions were also taken into account.

RESULTS

Patients

Twenty-seven patients were enrolled in the study and 26 patients were treated of which 19 have evaluable tumor assessment data. Specifically, 7 patients were treated in the 45 mg/m^2 arm, 3 in the 56.25 mg/m^2 , 7 in the 100 mg/m^2 , 2 in the 150 mg/m^2 , and 7 in 125 mg/m^2 arm. Seven patients had no tumor assessments beyond the baseline evaluation as a result of loss to follow-up (3 patients), patient request (1 patient), drug shortage (1 patient), and incomplete tumor evaluation (1 patient). All patients had discontinued therapy at the time of this analysis. Eighteen (69%) patients discontinued treatment because of disease progression, 4 (15%) due to adverse events (AEs) / toxicities, 2 (8%) for patient request, and 2 (8%) for drug shortage. Patient baseline demographics and characteristics were described in Table 1. Briefly, the median age was 60.5 years, with the majority of patients were male

(62%), Caucasian (81%), and had a baseline ECOG score of 1 (73%). The most common sites of primary tumor diagnosis were head and neck, colorectal, and kidney (12% each). Most patients had a carcinoma/adenocarcinoma (54%) and the rest had sarcoma. All patients had visceral metastases. The most common sites of metastases were lung/thoracic (69%), liver (46%), lymph node (42%), and abdomen/peritoneal (42%).

Treatment Exposure

For all patients, the median number of cycles administered was 3 (1–13), with 27% of patients having >3 cycles of therapy. The median cumulative rapamycin dose was 405 mg/m² (100–2200), with the median dose intensity of 68.9 mg/m²/week (11.4–150.0). At the MTD, the median number of cycles was also 3 (1–5), with the median cumulative dose of 800 mg/m² (100–900) and median dose intensity of 78.9 mg/m²/week (51.1–100.0).

Safety Results

Maximum Tolerated Dose—Following dose escalation to 100 mg/m², *nab*-rapamycin dose was initially escalated to 150 mg/m². Two DLTs occurred in the 150 mg/m² cohort: a grade 3 elevation of aspartate aminotransferase (AST) and a grade 4 thrombocytopenia. After observing DLTs at the 150 mg/m² cohort, a new dose level of 125 mg/m² was added for refinement of MTD. At the 125 mg/m² dose level, 2 DLTs occurred (grade 3 suicidal ideation and grade 3 hypophosphatemia); therefore, the MTD was reached and declared at 100 mg/m².

Treatment-related Adverse Events—For all cohorts and all grades, 25 of 26 (96%) patients experienced at least 1 TRAE. The most common nonhematologic TRAEs reported were mucosal inflammation (10 patients, 38%), fatigue (7 patients, 27%), rash (6 patients, 23%), diarrhea (6 patients, 23%), and nausea (5 patients, 19%, see Table 2). Most of these AEs were grade 1/2 events, with only 3 grade 3 nonhematologic AEs (2 elevated AST and 1 dyspnea). Specifically, at the MTD (100 mg/m²), all 7 patients experienced at least 1 TRAE of any grades, and the most common AEs were mucositis and fatigue (5 patients, 71% each). Four (15%) patients experienced at least 1 treatment-related serious AE, including arrhythmia (grade 2) and mood alteration (grade 3) both in the 125 mg/m² cohort, vomiting (grade 3) in the 45 mg/m² cohort, and dyspnea (grade 3) in the 100 mg/m² cohort.

The most common hematologic TRAE, for all cohorts and grades, were thrombocytopenia (58%), followed by hypokalemia (23%), anemia and hypophosphatemia (19% each), and neutropenia and hypertriglyceridemia (15% each, see Table 2). Most of these events were grade 1/2, and only 1 grade 4 hematologic event occurred (thrombocytopenia in the 150 mg/m² arm). At the MTD, the only hematologic AE was a grade 3 anemia.

Treatment-related Study Drug Reductions, Delays, and Discontinuations—Five (19%) patients experienced TRAEs that required study drug dose reductions and 50% of dose reductions occurred at cycle 2. Only 1 patient at the MTD had an AE that required a dose reduction, which occurred at cycle 4. The specific events requiring dose reductions were 1 grade 2 thrombocytopenia and 1 grade 2 dyslipidemia in the 100 mg/m² cohort, and 2 grade 3 thrombocytopenia and 1 grade 3 suicidal ideation in the 125 mg/m² cohort. The patient who experienced suicidal ideation had been on antidepressants prior to the trial. After the onset of grade 3 suicidal ideation (end of cycle 1), this patient received 2 cycles of *nab*-rapamycin at a reduced dose (100 mg/m²), during which no suicidal ideation was reported. Additionally, there was a dose reduction for a grade 2 elevated AST in the 45 mg/m² cohort. The dose was reduced to 30 mg/m² which was not specified in the protocol. This patient responded to treatment and the physician felt that continuing the treatment at a lower dose was in the best interest for this patient.

Sixteen (62%) patients had TRAEs requiring a dose delay: 4 (57%) patients in the 45 mg/m², 1 (33%) in the 56.25 mg/m², 4 (57%) in the 100 mg/m², 2 (100%) in the 150 mg/m², and 5 (71%) in the 125 mg/m² cohort. Specifically in the 100 mg/m² cohort, the treatment-related dose delays were due to 3 grade 2 thrombocytopenia, a grade 2 elevated triglycerides, a grade 2 mucosal inflammation, and a grade 3 dyspnea. Only 1 patient had a TRAE that resulted in study drug discontinuation (150 mg/m² cohort, 1 patient with a grade 4 thrombocytopenia and a grade 2 diarrhea).

Pharmacokinetics

Whole blood samples obtained during cycle 1 of treatment at the specified time points were analyzed for rapamycin concentration and non-compartmental pharmacokinetic analyses were performed. Of 27 enrolled patients, 26 were evaluable for pharmacokinetic analyses (patient demographics in Table 1). There was a rapid decline in whole blood levels of rapamycin in the first 2 hours following the 30-minute infusion of *nab*-rapamycin, which was followed by a slower elimination phase (Table 3 and Figure 1). The C_{max} increased proportionally over the dose range of 45–150 mg/m² as did the AUC, except for a relatively low AUC in the 125 mg/m² dose cohort (Table 3).

Efficacy Results

Of 19 patients evaluable for efficacy with best overall tumor response assessments, which included assessment of target, nontarget, and new lesions across all cycles, 1 patient in the 45 mg/m² cohort diagnosed with adenocarcinoma of the kidney and with bone and intrathoracic metastases had a confirmed PR. The target lesion of this patient was reduced by 35.1% and the duration of response lasted 183 days. Two (11%) patients had an overall tumor evaluation of SD (confirmed): 1 patient with mesothelioma had SD for 365 days and 1 patient with a neuroendocrine tumor in the left axillary node had SD for 238 days. Eight patients had SD that could not be confirmed either due to absence of follow-up tumor evaluation after the first SD, or due to progression after the first SD.

The waterfall plot in Figure 2 illustrates the percent change in the target tumors in patients with various tumor types and histologies. Two (11%) patients with adenocarcinoma of the kidney had >30% decrease in the target lesion, which included the patient dosed at 45 mg/m² (the patient mentioned above who had a confirmed PR) and another patient in the 56.25 mg/m² cohort, whose target lesion was reduced by 34.7% and a duration of response lasting 104 days. As seen in Figure 2, 13 (72%) patients had a target lesion objective response evaluation of SD. These patients had cancer of the bladder, colorectal, esophagus, head and neck, prostate, retroperitoneal, or uterus. Additionally, 3 (17%) patients had target lesion objective response of PD. As seen in Figure 2, patients with any decrease in the target tumor lesion had carcinoma/adenocarcinoma of the kidney, bladder, esophagus, or neuroendocrine cancer.

Of note, the molecular analyses of the tumor biopsy obtained from the patient with mesothelioma achieving the longest clinical benefit revealed no loss in phosphatase and tensin homolog (PTEN), or an activating mutation in *PIK3CA* or *AKT*. However, a single-nucleotide polymorphism was observed on *PHLPP2* (PH domain leucine-rich repeat protein phosphatase 2), a gene that codes for a protein phosphatase that mediates dephosphorylation of serine 473 in Akt1 (33).

Pharmacodynamics

PBMCs were collected from 18 patients. The effect of *nab*-rapamycin on mTOR signaling was assessed by evaluating the phosphorylation of mTOR targets, including 4EBP and S6K, and S6K target ribosomal S6 on phosphorylation sites, with 2 different assays (MSD and

RPPA) previously used to demonstrate rapamycin-mediated inhibition of downstream signaling (34). The MSD phosphoprotein assays revealed very low baseline expression of S6 S240/244 and 4EBP T37/46 in pretreatment PBMC samples, thus we were unable to assess regulation by *nab*-rapamycin treatment. Therefore, the effect of *nab*-rapamycin on the functional proteomic profile was assessed by RPPA.

nab-Rapamycin treatment was associated with a significant decrease of S6K T389 on D2 with persistent inhibition at D8 (Figure 3A and 3B) at all doses. *nab*-Rapamycin treatment was associated with significant decrease of 4EBP1 T70 levels on D2 and D4, but with recovery by D8 (Figure 3A and 3C). *nab*-Rapamycin at 56.25 dose level was not associated with a decrease in 4EBP1 T70 levels, while a significant decrease in 4EBP1 T70 levels was seen with higher doses (Figure 3B). These results demonstrate that *nab*-rapamycin has a dose-dependent effect on mTOR signaling, with pathway inhibition being seen at 56.25 mg/m² and higher doses. The duration of inhibition differs between downstream targets, and is longer for S6K T389 than for 4EBP1 T70.

Next, we determined whether the PK data correlated with pathway inhibition on RPPA. There was a moderate negative correlation between PBMC 4EBP1 T70 levels and serum rapamycin concentrations ($r^2 = -0.446$) as well as between S6K T389 and serum rapamycin concentration ($r^2 = -0.517$). Unfortunately, PBMCs were not available for the patient who had a PR. There was no significant difference in the inhibition 4EBP1 and S6K phosphorylation between patients who had SD and patients who had PD.

DISCUSSION

The results of this phase 1 dose-finding study demonstrated that the MTD for *nab*-rapamycin in patients with advanced nonhematologic malignancies was 100 mg/m², which produced favorable safety profile without the DLTs typically observed with rapalogs. It is notable that most of the DLTs such as mucositis/stomatitis that are observed with other mTOR inhibitors (35, 36) were not dose-limiting with *nab*-rapamycin: all mucositis events in this study were grade 1/2. mTOR inhibitors can produce potentially life-threatening pneumonitis (2), but no pneumonitis was observed in this study. It is notable that 27% of patients were ≥ 65 years old, a frail population that is more prone to toxicities and potentially receive less benefits than younger patients from treatment with everolimus/temsirolimus (3). Additionally, dermatologic toxicities such as rash, which were reported to occur in up to 50% of patients receiving temsirolimus (36), occurred in only 6 patients (23%) receiving *nab*-rapamycin and were mild (grade 1 in 4 patients) or moderate in nature (grade 2 in 2 patients). Since *nab*-rapamycin contains human albumin, it is possible that the skin reactions are in part related to the biologic component of the product. In humans, the known toxicities with rapamycin and other rapalogs are hypercholesterolemia, hypertriglyceridemia, lymphopenia, thrombocytopenia, mucositis, arthralgia, and infections (35–37). In this study, only a few grade ≥ 3 TRAEs were observed in this heavily-pretreated incurable patient population, with the most common events being thrombocytopenia, anemia, hypophosphatemia, and elevated AST. Suicidal ideation, which was a DLT in this study, is not a common adverse event seen with mTOR inhibitors, although cancer patients are in general at an increased risk of suicide than the general population. Recent reports showed evidence that upregulated mTOR in rat hippocampus had an antidepressant effect, indicating that mTOR inhibition could potentially contribute to depression (38). A phase 2 study with temsirolimus also reported depression in 5% of patients, with 10% grade 3 or 4 depression at 250 mg dose level (39). Depression was also reported as a DLT with a pan-PI3K inhibitor (BKM120) given in combination with letrozole in post-menopausal women with metastatic breast cancer (40). With *nab*-rapamycin at the MTD, only 1 patient had a grade 3 hematologic event (anemia) and 1 patient had a grade 3 nonhematologic event (dyspnea),

supporting the tolerability of *nab*-rapamycin in this frail population. Overall, *nab*-rapamycin was well tolerated at the MTD displayed in this phase 1 study making it a promising candidate to explore in further clinical trials.

The patient with overall tumor response evaluation of PR had renal cell carcinoma, a disease in which mTOR overactivity has been described (41, 42) and for which temsirolimus was first approved. Similarly, the longest clinical benefit was observed in a patient with mesothelioma and a patient with neuroendocrine tumor, diseases in which mTOR overactivity has also been described (43). It was expected that patients with tumors that have activation of the PI3K/AKT/mTOR pathway may benefit most from *nab*-rapamycin treatment. In accordance with this expectation, molecular analysis of the tumor biopsy from the patient with mesothelioma revealed a single-nucleotide polymorphism that has been associated with PI3K/AKT/mTOR pathway activation (30, 33). The modest response rate observed in the study with most patients having SD has been observed with other rapalogs as well, including everolimus, temsirolimus, and ridaforolimus (deforolimus) (35, 36, 44, 45).

The pharmacokinetic analysis identified a somewhat proportional increase of C_{max} and AUC with increasing dose of *nab*-rapamycin. At the MTD of 100 mg/m², the C_{max} of *nab*-rapamycin was 3227.61 ng/mL, which is significantly higher than C_{max} of rapamycin levels achieved in other studies: the C_{max} of 15.5 ng/mL reported with 5 mg oral rapamycin (45), C_{max} of 37.9 ng/mL reported with 9 mg oral rapamycin (46), C_{max} of 57.72 ng/mL reported with 60 mg oral rapamycin (47), and day 5 rapamycin C_{max} of 133.9 ng/mL after 5 days of IV daily administration of temsirolimus at 19.1 mg/m² (48). As expected, the half-life of rapamycin from *nab*-rapamycin was relatively long at 40–91 hours across the tested dose range and was 63 hours at the MTD of 100 mg/m², similar to the half-life of 61–69 hours for oral rapamycin and IV temsirolimus and 45–52 hour for deferolimus given by weekly IV infusion (35, 36, 49, 50). Together the favorable safety profile and similar efficacy and PK profile of *nab*-rapamycin compared historically with other rapalogs administered as a weekly IV infusion indicate that *nab*-rapamycin can also be given on a weekly IV schedule (35, 36, 49). Of the known rapalogs, temsirolimus is administered once weekly IV, but requires antihistamine and steroid premedication (51, 52). Rapamycin is generally administered on a flat dosing (on a fixed mg-basis) schedule instead of on a body surface area (BSA) normalized basis (45, 46, 51). While the analysis of BSA versus flat dosing was not conducted, it is possible that *nab*-rapamycin could also be administered by flat dosing.

nab-Rapamycin significantly inhibited mTOR targets S6K and 4EBP1 in the present study. There may be differences between the pharmacodynamics of mTOR inhibition of these 2 targets. Others have also shown differential inhibition of S6K and 4EBP1 by rapamycin, with potent inhibition of S6K throughout the treatment, but recovery of phosphorylation of 4EBP1 within 6 h after an initial inhibition (53), indicating a differential mechanistic effect of rapamycin on S6K and 4EBP1, which may also be cell-type specific. Importantly, at doses of 56.25 mg/m² and above, the suppression of S6K T389 and 4EBP1 T70 was maintained for several days after administration suggesting that weekly dosing of *nab*-rapamycin at the established MTD (100 mg/m²) may be adequate in suppressing these relevant biological targets.

mTOR is located downstream of numerous other therapeutic targets such as PI3K and AKT, VEGF, VEGF receptor tyrosine kinases, which provides opportunities for combination therapy for mTOR inhibitors to potentially increase their therapeutic index. The combination of *nab*-rapamycin with AKT inhibitor, perifosine, has shown remarkable activity in preclinical models of multiple myeloma (27) and breast cancer (20), but these results are yet to be confirmed in clinical settings. Several clinical studies are currently investigating

bevacizumab combined with everolimus (54) or temsirolimus (55) in RCC. Studies of either sunitinib or sorafenib in combination with mTOR inhibitors, such as rapamycin (NCT00555256), (56), temsirolimus (NCT01122615) or everolimus (NCT00422344) have been promising, however, the added toxicity of combination therapies may be concerning (57). This may suggest that dose level, administration route, and/or dosing schedules may not be optimal in the clinic. The encouraging safety and pharmacokinetic profile of single agent *nab*-rapamycin in this phase 1 study indicate that *nab*-rapamycin may be well suited for targeted combination therapies on a weekly IV schedule.

Taken together, *nab*-rapamycin as single agent was well tolerated with preliminary evidence of response and SD in this difficult-to-treat patient population with unresectable advanced nonhematologic malignancies, and should be considered for further study both as single agent and in synergistic combinations.

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Translational Relevance

In this first clinical evaluation of nanoparticle human albumin-bound rapamycin (*nab*-rapamycin), an mTOR inhibitor, was well tolerated given intravenously in patients with unresectable advanced solid tumors. Most rapalogs are oral preparations requiring toxic surfactants for intravenous formulation due to poor water solubility. Dose-limiting toxicities including mucositis/stomatitis that were observed with mTOR inhibitors (1) were not dose-limiting with *nab*-rapamycin. mTOR inhibitors can produce potentially life-threatening pneumonitis (2), yet no pneumonitis was observed in this study. Notably, 27% of patients were ≥ 65 years old, a frail population that are more prone to toxicities and receive lesser benefits than younger patients from everolimus/temsirolimus (3).

Preliminary proof of efficacy was observed. One patient with partial response had kidney adenocarcinoma and 2 patients with the longest clinical benefit had mesothelioma and neuroendocrine tumor; in all 3 cancer types mTOR overactivity has been described. *nab*-Rapamycin produced a fairly dose-proportional C_{max} and AUC increase, and significantly inhibited mTOR targets.

Figure 1.
nab-Rapamycin plasma concentration by time

Figure 2. Waterfall plot showing percent change of target lesion from baseline by patient and tumor types

Figure 3.

Regulation of mTOR signaling in PBMCs. A) Levels of 4EBP1 T70, S6K T389, S6 S235/236 and S6 S240/244 were assessed during cycle 1 of nab-rapamycin treatment, on D1(pre-treatment), D2, D4 and D8 (trough). Protein expression is expressed in Log₂ scale. Red line depicts average expression levels. B) Expression of S6K T389 in PBMCs at different timepoints in different dose cohorts. C) Expression of 4EBP1 T70 in PBMCs at different timepoints in different dose cohorts.

Table 1

Baseline patient demographics and characteristics

	MTD n = 7	All Treated Patients n = 26
Age, median years (range)	57 (36, 76)	60.5 (18, 78)
<65 years old, n (%)	4 (57)	19 (73)
65 years old, n (%)	3 (43)	7 (27)
Gender, n (%)		
Male	5 (71)	16 (62)
Female	2 (29)	10 (38)
Race		
Asian, n (%)	0	1 (4)
African Heritage, n (%)	0	2 (8)
Caucasian, n (%)	6 (86)	21 (81)
Hispanic, Latino, n (%)	1 (14)	2 (8)
ECOG, n (%)		
0	2 (29)	5 (19)
1	5 (71)	19 (73)
2	0	2 (8)
Stage at Current Diagnosis, n (%)	7 (100)	26 (100)
IV		
Site of Primary Diagnosis, n (%)		
Bladder	1 (14)	1 (4)
Breast	0	1 (4)
Colorectal	2 (29)	3 (12)
Esophagus	1 (14)	2 (8)
Head and Neck	1 (14)	3 (12)
Kidney	0	3 (12)
Lung/Thoracic	1 (14)	2 (8)
Prostate	0	1 (4)

	MTD n = 7	All Treated Patients n = 26
Stomach	0	1 (4)
Uterus	1 (14)	1 (4)
Other	0	8 (31)

Histology of Primary Diagnosis, n (%)

Carcinoma / Adenocarcinoma	5 (71)	14 (54)
Sarcoma / Sarcomatoid	2 (29)	12 (46)

Site of Metastasis, n (%)

Visceral	7 (100)	26 (100)
Nonvisceral	0	0

Abbreviations: ECOG, Eastern Cooperative Oncology Group; MTD, maximum tolerated dose

Treatment-related grade 1–4 hematologic and nonhematologic adverse events reported in 10% of all treated patients

Table 2

MTD (100 mg/m ²) n = 7													All Treated Patients n = 26			
NCI CTCAE v 3.0	G1	G2	G3	G4	G1	G2	G3	G4	G1	G2	G3	G4				
Hematologic AEs, n (%)																
Anemia	0	0	1 (14)	0	0	3 (12)	2 (8)	0								
Hypokalemia	1 (14)	0	0	0	5 (19)	0	1 (4)	0								
Hypophosphatemia	0	1 (14)	0	0	1 (4)	2 (8)	2 (8)	0								
Hypertiglyceridemia	1 (14)	1 (14)	0	0	2 (8)	1 (4)	1 (4)	0								
Neutropenia	1 (14)	0	0	0	2 (8)	1 (4)	1 (4)	0								
Thrombocytopenia	1 (14)	4 (57)	0	0	5 (19)	6 (23)	3 (12)	1 (4)								
Nonhematologic AEs, n (%)																
AST/SGOT	0	0	0	0	1 (4)	0	2 (8)	0								
Constipation	0	1 (14)	0	0	1 (4)	2 (8)	0	0								
Diarrhea	1 (14)	0	0	0	3 (12)	3 (12)	0	0								
Dyspnea	0	1 (14)	1 (14)	0	1 (4)	2 (8)	1 (4)	0								
Fatigue	1 (14)	4 (57)	0	0	1 (4)	6 (23)	0	0								
Infection, oral cavity	1 (14)	1 (14)	0	0	3 (12)	2 (8)	0	0								
Mucositis/stomatitis	3 (43)	2 (29)	0	0	7 (27)	3 (12)	0	0								
Nausea	1 (14)	1 (14)	0	0	3 (12)	2 (8)	0	0								
Rash	1 (14)	0	0	0	4 (15)	2 (8)	0	0								
Weight loss	0	0	0	0	1 (4)	2 (8)	0	0								

Abbreviations: AE, adverse event; AST/SGOT, aspartate aminotransferase / serum glutamic oxaloacetic transaminase; G, grade; MTD, maximum tolerated dose; NCI CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events

Summary statistics of pharmacokinetic variable estimates

Table 3

Dose, mg (n)	HL, h (%CV)	t _{max} , h (%CV)	C _{max} , ng/mL (40.30)	C _{max} /dose, ng•m ² /mL/mg (%CV)	AUC _{inf} , ng•h/mL (18.74)	AUC _{inf} /dose, ng•h ² •m ² /mL/mg (%CV)	CL, mL/min/m ² (%CV)	V _{ss} , L/m ² (%CV)
45 (7)	39.57 (26.79)	0.46 (20.27)	1776.27 (40.30)	39.47 (40.30)	24564.19 (18.74)	545.87 (18.74)	31.29 (15.34)	107.36 (11.35)
56.25 (3)	46.31 (33.36)	0.44 (21.66)	2881.77 (36.38)	51.23 (36.38)	25600.46 (30.01)	455.12 (30.01)	39.35 (34.88)	135.70 (25.71)
100 (7)	63.10 (35.77)	0.46 (20.35)	3227.61 (38.00)	32.28 (38.00)	44072.64 (40.31)	440.73 (40.31)	44.34 (44.72)	215.09 (29.03)
125 (7)	54.93 (22.74)	0.36 (37.42)	4261.24 (41.01)	34.09 (41.01)	27423.48 (21.34)	219.39 (21.34)	78.80 (20.05)	296.74 (20.79)
150 (2)	90.75 (5.42)	1.10 (72.86)	3828.45 (96.39)	25.52 (96.39)	69400.48 (2.25)	462.67 (2.25)	36.03 (2.25)	211.11 (41.62)

Note: mean values are presented for all variables

Abbreviations: AUC_{inf}, area under the curve with concentration extrapolated; CL, plasma clearance; C_{max}, peak plasma concentration; HL, half-life; t_{max}, time at peak plasma concentration; V_{SS}, volume of distribution at steady state