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

Ana M. Gonzalez-Angulo1, Funda Meric-Bernstam2,3, Sant Chawla4, Gerald Falchook3, David Hong3, Argun Akcakanat6, Huichin Chen1, Aung Naing3, Siqing Fu3, Jennifer Wheeler3, Stacy Moulder1, Thorunn Helgason3, Shaoyi Li6, Ileana Elias6, Neil Desai6, and Razelle Kurzrock5

Abstract

Purpose: This dose-finding phase I study investigated the maximum-tolerated dose (MTD) and safety of weekly nanoparticle albumin-bound rapamycin (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 45 mg/m2. Additional doses were 56.25, 100, 150, and 125 mg/m2.

Results: Of 27 enrolled patients, 26 were treated. Two dose-limiting toxicities (DLT) occurred at 150 mg/m2 [grade 3 aspartate aminotransferase (AST) elevation and grade 4 thrombocytopenia], and two DLTs occurred at 125 mg/m2 (grade 3 suicidal ideation and grade 3 hypophosphatemia). Thus, the MTD was declared at 100 mg/m2. Most treatment-related adverse events (TRAE) 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 one grade 3 nonhematologic TRAE (dyspnea) and one grade 3 hematologic event (anemia) occurred at the MTD. One patient with kidney cancer had a partial response and 2 patients remained on study for 365 days (patient with mesothelioma) and 238 days (patient with neuroendocrine tumor). The peak concentration (Cmax) and area under the concentration–time curve (AUC) of rapamycin increased with dose between 45 and 150 mg/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 weekly (3 of 4 weeks) given intravenously, which was well tolerated with preliminary evidence of response and stable disease, and produced a fairly dose-proportional pharmacokinetic profile in patients with unresectable advanced nonhematologic malignancies. Clin Cancer Res; 19(19); 5474–84. ©2013 AACR.

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 phosphoinositide 3-kinase (PI3K)/Akt pathway, and a key regulator of cell survival, proliferation, stress, and metabolism (1). mTOR inhibition with rapamycin and rapalogs (everolimus and temsirolimus) has proven to be effective in various solid tumors including renal cell carcinoma, neuroendocrine tumors, and breast cancer (2–12).

Although rapamycin is an efficacious allosteric inhibitor of mTOR complex 1 (mTORC1), it has low oral bioavailability, poor solubility, and dose-limiting intestinal toxicity (13, 14). Other rapalogs, including everolimus and ridaforolimus, are also oral preparations and are often associated with significant stomatitis (15). Temsirolimus, a prodrug of rapamycin, requires conversion by the CYP3A enzyme and also carries a significant risk for developing skin rash and stomatitis (16). Because none of the rapalogs are highly water soluble, they require surfactants and solvents in an intravenous formulation, such as polysorbate 80 for temsirolimus (17). 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 (17). The nanoparticle albumin-bound rapamycin (nab-rapamycin; Celgene Inc.),

Notes:
- Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).
- Corresponding Author: Ana M. Gonzalez-Angulo, Department of Breast Medical Oncology and Department of Systems Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas; sarcoma Oncology Center, Santa Monica; Division of Hematology–Oncology, University of California, San Diego, California; and Celgene, Summit, New Jersey.
with a mean particle size of about 100 nm, is freely dispersible in saline and is suitable for intravenous administration.

Patients and Methods

This study was conducted at MD Anderson Cancer Center (Houston, 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 (25). Written informed consent was obtained from all patients before study initiation.

Patients

Eligible patients were 18 years or older, had histologically or cytologically confirmed diagnosis of stage IV cancer that 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 or more 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 before 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 biologic composition.

Study design

This dose-finding study evaluated MTD and dose-limiting toxicities (DLT) 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 on the basis of 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. The protocol was amended to ensure that all patients at a given dose level complete one cycle of therapy before patients were enrolled at the next dose level. If no DLT was observed, 3 additional patients were enrolled at the next dose level. If one DLT was observed, the dose level was expanded to 6 patients. If two DLTs were observed at a given dose level, the MTD was considered to be exceeded. Of the 6-patient expanded cohort, if ≤1 of 6 patients experienced a DLT, this was defined as the MTD. All patients at a given dose level completed one 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 (i.e., without fever or infection) lasting more than 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 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 one dose of study drug (treated population) were evaluated for safety. Safety and tolerability endpoints included the incidence of treatment-related adverse events (TRAE) 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 exploratory efficacy analysis included the summary of percentage of patients who achieved an objective with confirmed complete or partial tumor response (CR or PR, respectively) and the percentage of patients with confirmed stable disease 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 carried out 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 PTEN loss was carried out with immunohistochemistry (IHC) using monoclonal mouse anti-human PTEN antibody clone 6H2.1 from Dako at 1:100 dilution, as described by Gonzalez-Angulo and colleagues (26). 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 × percentage of positive cells. Staining intensity was determined as 0, negative; 1, weak; 2, moderate; and 3, strong. Percentage of positive cells 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 spectrometry-based approach evaluating single-nucleotide polymorphisms (SNP) was used to detect known mutations in members of the PI3K pathway. Molecular analysis was conducted in patients who showed 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, 0.5, 2, 4, 6, 8, 24, 48, 72, 96, and 168 hours. The samples were stored frozen at a temperature between −20°C and −80°C until shipment for analysis to St. George’s Hospital at the University of London (London, United Kingdom).

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 2,000 ng/mL with 32-desmethoxyrapamycin used as an internal standard. Analytes were extracted using a solvent mixture and detected and quantified by reverse phase HPLC 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 ($C_{\text{max}}$), half-life (t1/2), area under the concentration–time curve (AUC), clearance (Cl), and steady-state volume of distribution (Vss). A simple regression model was applied to assess the relationship of the pharmacokinetic parameters with dose.

Peripheral blood mononuclear cells and reverse phase protein arrays

Whole blood for pharmacodynamics evaluation was collected only during cycle 1 at four time points: C1 D1 (pretreatment), C1 D2, C1 D4, and C1 D8 (immediately before next dose) in an 8-mL cell preparation tube with sodium citrate (Becton, Dickinson and Company). Separation of peripheral blood mononuclear cells (PBMC) from whole blood was accomplished through density gradient centrifugation using Ficoll following the 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 (RPPA) was conducted in the MD Anderson Cancer Center Functional Proteomics RPPA Core Facility as described previously (27). PBMC samples were resuspended in RPPA lysis buffer containing 0.25% sodium deoxycholate. Protein concentrations were determined using BCA method (Pierce) and 4 × SDS sample buffer was added. Final protein concentration was adjusted to 3 μg/μL. 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 Meso Scale discovery (MSD) phosphoprotein assays (Meso Scale Discovery).

The RPPA spot signal intensity data obtained from MicroVigene automated RPPA module (VigeneTech, Inc.) were analyzed using the R package SuperCurve (version 1.4.3; ref. 28), 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- versus posttreatment and inhibition effects at each dose level and each pair of time points. Tukey tests were also used for pairwise comparisons. To test the association of proteins expression on patients’ response, patients with stable disease and progressive disease were also compared using logistic models adjusted by time points. 

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/m² arm, 1 additional patient was added after a patient did not complete a full cycle, 3 in the 56.25 mg/m², 7 in the 100 mg/m², 2 in the 150 mg/m², and 7 in 125 mg/m² 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/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, and 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 three (1–11, 15, 29), with 27% of patients having more than three cycles of therapy. The median cumulative rapamycin dose was 405 mg/m² (100–2,200), with the median dose intensity of 68.9 mg/m²/wk (11.4–150.0). At the MTD, the median number of cycles was also three (1–3, 15, 29), with the median cumulative dose of 800 mg/m² (100–900) and median dose intensity of 78.9 mg/m²/wk (51.1–100.0).

Safety results

MTD. 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, two DLTs occurred (grade 3 suicidal ideation and grade 3 hypophosphatemia); therefore, the MTD was reached and declared at 100 mg/m².

Table 1. Baseline patient demographics and characteristics

<table>
<thead>
<tr>
<th></th>
<th>MTD</th>
<th>All treated patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Age, median years (range)</td>
<td>57 (36, 76)</td>
<td>60.5 (18, 78)</td>
</tr>
<tr>
<td>&lt;65 years, n (%)</td>
<td>4 (57)</td>
<td>19 (73)</td>
</tr>
<tr>
<td>≥65 years, n (%)</td>
<td>3 (43)</td>
<td>7 (27)</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (71)</td>
<td>16 (62)</td>
</tr>
<tr>
<td>Female</td>
<td>2 (29)</td>
<td>10 (38)</td>
</tr>
<tr>
<td>Race, n (%)</td>
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<td></td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>African heritage</td>
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<tr>
<td>Caucasian</td>
<td>6 (86)</td>
<td>21 (81)</td>
</tr>
<tr>
<td>Hispanic, Latino</td>
<td>1 (14)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>ECOG, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2 (29)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>1</td>
<td>5 (71)</td>
<td>19 (73)</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Stage at current diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>7 (100)</td>
<td>26 (100)</td>
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<td>Site of primary diagnosis, n (%)</td>
<td></td>
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<tr>
<td>Bladder</td>
<td>1 (14)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Breast</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>2 (29)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1 (14)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Head and neck</td>
<td>1 (14)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Lung/thoracic</td>
<td>1 (14)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Prostate</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Uterus</td>
<td>1 (14)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>8 (31)</td>
</tr>
<tr>
<td>Histology of primary diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma/adenocarcinoma</td>
<td>5 (71)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>Sarcoma/sarcomatoid</td>
<td>2 (29)</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Site of metastasis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral</td>
<td>7 (100)</td>
<td>26 (100)</td>
</tr>
<tr>
<td>Nonvisceral</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
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TRAEs. For all cohorts and all grades, 25 of 26 (96%) patients experienced at least one 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 adverse events were grade 1/2 events, with only three grade 3 nonhematologic adverse events (two elevated AST and one dyspnea). Specifically, at the MTD (100 mg/m²), all 7 patients experienced at least one TRAE of any grade, and the most common adverse events were mucositis and fatigue (5 patients; 71% each). Four (15%) patients experienced at least one treatment-related serious adverse event, 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 one grade 4 hematologic event occurred (thrombocytopenia in the 150 mg/m² arm). At the MTD, the only hematologic adverse event 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 adverse event that required a dose reduction, which occurred at cycle 4. The specific events requiring dose reductions were one grade 2 thrombocytopenia and one grade 2 dyslipidemia in the 100 mg/m² cohort, and two grade 3 thrombocytopenia and one grade 3 suicidal ideation in the 125 mg/m² cohort. The patient who experienced suicidal ideation had been on antidepressants before the trial. After the onset of grade 3 suicidal ideation (end of cycle 1), this patient received two cycles of nab-rapamycin at a reduced dose (100 mg/m²), during which no suicidal ideation was reported. In addition, 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 three 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

Table 2. Treatment-related grade 1–4 hematologic and nonhematologic adverse events reported in 10% or more of all treated patients

<table>
<thead>
<tr>
<th>NCI CTCAE v 3.0</th>
<th>MTD (100 mg/m²)</th>
<th>All treated patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1</td>
<td>G2</td>
</tr>
<tr>
<td>Hematologic AEs, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>1 (14)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1 (14)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Nonhematologic AEs, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Constipation</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (14)</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (14)</td>
<td>4 (57)</td>
</tr>
<tr>
<td>Infection, oral cavity</td>
<td>1 (14)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Mucositis/stomatitis</td>
<td>3 (43)</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1 (14)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (14)</td>
<td>0</td>
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<tr>
<td>Weight loss</td>
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</table>

Abbreviations: AE, adverse event; G, grade.
rapamycin concentration and noncompartmental pharmacokinetic analyses were conducted. 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 Fig. 1). The $C_{\text{max}}$ increased proportionally over the dose range of 45 to 150 mg/m$^2$ as did the AUC, except for a relatively low AUC in the 125 mg/m$^2$ 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 (5%) in the 45 mg/m$^2$ 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 stable disease (confirmed): 1 patient with mesothelioma had stable disease for 365 days and 1 patient with a neuroendocrine tumor in the left axillary node had stable disease for 238 days. Eight patients had stable disease that could not be confirmed either due to absence of follow-up tumor evaluation after the first stable disease, or due to progression after the first stable disease.

The waterfall plot in Fig. 2 illustrates the percentage change in the target tumors in 18 evaluable patients with various tumor types and histologies. Two (11%) patients with adenocarcinoma of the kidney had more than 30% decrease in the target lesion, which included the patient dosed at 45 mg/m$^2$ (the patient mentioned above who had a confirmed PR) and another patient in the 56.25 mg/m$^2$ cohort, whose target lesion was reduced by 34.7% and a duration of response lasting 104 days. As seen in Fig. 2, 13 (72%) patients had a target lesion objective response evaluation of stable disease. These patients had cancer of the bladder, colorectal, esophagus, head and neck, prostate, retroperitoneal, or uterus. It is notable that many of these patients with a target tumor evaluation of stable disease did not have a confirmed overall tumor evaluation of stable disease, which in addition to target lesions also accounted for nontarget and new lesions. In addition, 3 (17%) patients had target lesion objective response of progressive disease. As seen in Fig. 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 PTEN, or an activating mutation in PIK3CA or AKT. However, a SNP 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 (30).
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 two different assays (MSD and RPPA) previously used to show rapamycin-mediated inhibition of downstream signaling (31). 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 (Fig. 3A and B) at all doses. nab-Rapamycin treatment was associated with significant decrease of 4EBP1 T70 levels on D2 and D4, but with recovery by D8 (Fig. 3A and C). nab-Rapamycin at 56.25 mg/m² dose level was not associated with a decrease in 4EBP1 T70 levels, whereas a significant decrease in 4EBP1 T70 levels was seen with higher doses (Fig. 3B). These results show 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 pharmacokinetic 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 stable disease and patients who had progressive disease.

Discussion

The results of this phase I dose-finding study showed 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 (32, 33), 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 (34), but no pneumonitis was observed in this study; however, it is possible that the duration of treatment and small number of patients influenced this outcome. It is notable that 27% of patients were 65 years or older, a frail population that is more prone to toxicities and potentially receive less benefits than younger patients from treatment with everolimus/temsirolimus (29). In addition, dermatologic toxicities such as rash, which were reported to occur in up to 50% of patients receiving temsirolimus (33), 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). Because 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 (32, 33, 35). In this study, only a few grade 1-2 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 (36). A phase II study with temsirolimus also reported depression in 5% of patients, with 10% grade 3 or 4 depression at 250 mg dose level (37). Depression was also

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 (pretreatment), 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 time points in different dose cohorts. C, expression of 4EBP1 T70 in PBMCs at different time points in different dose cohorts.
reported as a DLT with a pan-Pi3K inhibitor (BKM120) given in combination with letrozole in postmenopausal women with metastatic breast cancer (38). 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 I 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 (39, 40) 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 (41). It is notable that the patient with a PR and the patient with the longest clinical benefit received 45 mg/m² nab-rapamycin, which suggests that nab-rapamycin was efficacious at the lowest dose tested and it may not be essential to treat patients at the MTD of 100 mg/m²; however, this needs to be investigated in further studies. 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 SNP that has been associated with PI3K/AKT/mTOR pathway activation (27, 30). The modest response rate observed in the study with most patients having stable disease has been observed with other rapalogs as well, including everolimus, temsirolimus, and ridaforolimus (deforolimust; refs. 32, 33, 42, 43).

The pharmacokinetic analysis identified a somewhat proportional increase of Cmax and AUC with increasing dose of nab-rapamycin. At the MTD of 100 mg/m², the Cmax of nab-rapamycin was 3,227.61 ng/mL, which is significantly higher than Cmax of rapamycin levels achieved in other studies: the Cmax of 15.5 ng/mL reported with 5 mg oral rapamycin (43), Cmax of 37.9 ng/mL reported with 9 mg oral rapamycin (44), Cmax of 57.72 ng/mL reported with 60 mg oral rapamycin (45), and day 5 rapamycin Cmax of 133.9 ng/mL after 5 days of intravenous daily administration of temsirolimus at 19.1 mg/m² (46). As expected, the half-life of rapamycin from nab-rapamycin was relatively long at 40 to 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 to 69 hours for oral rapamycin and intravenous temsirolimus and 45 to 52 hours for deferolimus given by weekly intravenous infusion (32, 33, 47, 48). Together, the favorable safety profile and similar efficacy and pharmacokinetic profile of nab-rapamycin compared historically with other rapalogs administered as a weekly intravenous infusion indicate that nab-rapamycin can also be given on a weekly intravenous schedule (32, 33, 47). Of the known rapalogs, temsirolimus is administered once weekly intravenously, but requires antihistamine and steroid premedication (49, 50). Rapamycin is generally administered on a flat dosing (on a fixed mg-basis) schedule instead of on a body surface area (BSA)–normalized basis (43, 44, 49). Although 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 two 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 hours after an initial inhibition (51), 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 biologic 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 an AKT inhibitor has shown remarkable activity in preclinical models of multiple myeloma (23) and breast cancer (16), but these results are yet to be confirmed in clinical settings. Clinical studies are currently investigating bevacizumab combined with everolimus in RCC (52). Studies of either sunitinib or sorafenib in combination with mTOR inhibitors, such as rapamycin (NCT00555256; ref. 53) or everolimus (NCT00422344) have been promising, however, the added toxicity of combination therapies may be concerning (54). 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 I study indicate that nab-rapamycin may be well suited for targeted combination therapies on a weekly intravenous schedule.

Taken together, nab-rapamycin as single agent was well tolerated with preliminary evidence of response and stable disease 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.

Disclosure of Potential Conflicts of Interest

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Authors’ Contributions

Conception and design: A.M. Gonzalez-Angulo, F. Meric-Bernstam, I. Elias, N. Desai, R. Kurzrock
Development of methodology: A.M. Gonzalez-Angulo, F. Meric-Bernstam, N. Desai, R. Kurzrock

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.M. Gonzalez-Angulo, F. Meric-Bernstam, G. Falchook, D. Hong, A. Aclakanat, A. Naing, S. Fu, J. Wheler, S. Moulder, T. Helgason, N. Desai, R. Kurzrock

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.M. Gonzalez-Angulo, F. Meric-Bernstam, G. Falchook, H. Chen, S. Fu, S. Moulder, T. Helgason, S. Li, N. Desai, R. Kurzrock

Writing, review, and/or revision of the manuscript: A.M. Gonzalez-Angulo, F. Meric-Bernstam, S. Chaula, G. Falchook, D. Hong, A. Naing, S. Fu, J. Wheler, S. Moulder, T. Helgason, S. Li, J. Elias, N. Desai, R. Kurzrock


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References


17. Pfizer. Prescribing Information: TORISEL Kit (temsirolimus) injection, for intravenous infusion only; 2011.


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Ana M. Gonzalez-Angulo, Funda Meric-Bernstam, Sant Chawla, et al.


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