Phase I Trial of Encapsulated Rapamycin in Patients with Prostate Cancer Under Active Surveillance to Prevent Progression

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ABSTRACT

No approved medical therapies prevent progression of low-grade prostate cancer. Rapamycin inhibits cell proliferation and augments immune responses, producing an antitumor effect. Encapsulated rapamycin (eRapa) incorporates rapamycin into a pH-sensitive polymer, ensuring consistent dosing. Here, we present results from a phase I trial evaluating the safety and tolerability of eRapa in patients with prostate cancer. Patients with Gleason ≤7 (3+4) disease (low and intermediate risk) under active surveillance were enrolled in a 3+3 study with three eRapa dosing cohorts (cohort 1, 0.5 mg/week; cohort 2, 1 mg/week; and cohort 3, 0.5 mg/day). Patients were treated for 3 months and followed for an additional 3 months to assess safety, pharmacokinetics, quality of life (QoL), immune response, and disease progression. Fourteen patients (cohort 1, $n = 3$; cohort 2, $n = 3$; and cohort 3, $n = 8$) were enrolled. In cohort 3, one dose-limiting toxicity (DLT; neutropenia) and two non-DLT grade 1–2 adverse events (AE) occurred that resulted in patient withdrawal. All AEs in cohorts 1 and 2 were grade 1. Peak serum rapamycin concentration was 7.1 ng/mL after a 1 mg dose. Stable trough levels (∼2 ng/mL) developed after 48–72 hours. Daily dosing mildly worsened QoL, although QoL recovered after treatment cessation in all categories, except fatigue. Weekly dosing increased naïve T-cell populations. Daily dosing increased central memory cell populations and exhaustion markers. No disease progression was observed. In conclusion, treatment with eRapa was safe and well-tolerated. Daily dosing produced higher frequencies of lower grade toxicities and transient worsening of QoL, while weekly dosing impacted immune response. Future studies will verify clinical benefit and long-term tolerability.

Prevention Relevance: There is an unmet medical need for a well-tolerated treatment capable of delaying progression of newly diagnosed low-grade prostate cancer. This treatment would potentially obviate the need for future surgical intervention and improve the perception of active surveillance as a more acceptable option among this patient population.

Introduction

Prostate cancer is the most common cancer among males in the United States, accounting for 21% of new cancer diagnoses and 10% of cancer-related deaths in 2020 (1). The vast majority of these newly diagnosed prostate cancers are low grade and early stage at the time of diagnosis (2). Active surveillance with digital rectal exams, PSA levels, and prostate biopsies is the preferred management of patients with low- and very low–risk disease and is an accepted management option for patients with favorable intermediate-risk disease (3). This management option reduces overtreatment while still closely monitoring for disease progression. Active surveillance is safe, with a <1% risk of prostate cancer at 10 and 15 years after diagnosis among low-risk patients (4–7). Patients with Gleason 7 (3+4) disease are at higher risk for intervention (5-year treatment-free survival of 49% in Gleason 3+4 vs. 64% in Gleason 3+3 disease; ref. 8), but select patients can still be appropriate candidates for active surveillance (9, 10).

Despite the demonstrable safety record of active surveillance, this treatment strategy has low acceptance among the applicable patient population (11–13). Even among patients who initially select an active surveillance strategy, up to 20% discontinue active surveillance within 5 years of initiation due to personal preference, rather than evidence of progression (14). Particularly among younger patients, the prospect of not actively treating a known cancer is associated with feelings of anxiety and uncertainty (15). As no medical therapies have been shown to decrease the rate of disease progression, patients must choose between active surveillance and the more aggressive options of

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surgery or radiation (3). The addition of a well-tolerated medication that is capable of delaying progression would thus address an unmet medical need, obviating the need for future surgical intervention and improving the perception of active surveillance as a more acceptable option (16).

One such potential medication to prevent disease progression is rapamycin, an inhibitor of mTOR. Rapamycin has known antineoplastic activity across a broad range of malignancies (17–19) and has been shown *in vivo* to inhibit the progression of prostatic intraepithelial neoplasia (20). Furthermore, chronic low-dose rapamycin reduced the rates of age-related diseases and extend lifespan in preclinical mouse models (21–23) by reversing the effects of aging on the immune system and reducing T-cell exhaustion phenotypes (24, 25). However, current oral formulations of rapamycin are hindered by variable rates of absorption and bioavailability that limit prolonged use without therapeutic drug monitoring (26–28).

Encapsulated rapamycin (eRapa) is a novel formulation of rapamycin that incorporates submicron rapamycin particles into a pH-sensitive poly (methyl methacrylate) polymer (Eudragit L 100/S 100). This formulation improves bioavailability and allows for more consistent and stable dosing. We hypothesized that treatment with lower doses of eRapa, 0.5 and 1 mg, given in either weekly or daily dosing schedules would be safe and well-tolerated in patients with low- and intermediate-grade prostate cancer undergoing active surveillance.

Materials and Methods

Study design and objectives

This study was a phase I, single-center, open-label, dose escalation study of eRapa in patients with Gleason 6 and 7 (3+4) prostate cancer in an active surveillance program. After institutional review board approval, patients were recruited from a single tertiary center, University of Texas Health San Antonio (San Antonio, TX), from August 2018 to June 2019. Patients were given the option for enrollment after detailed discussion of the study and completion of informed written consent.

The primary objectives of this study were an assessment of safety and tolerability of eRapa. Secondary objectives included assessments of eRapa pharmacokinetics, immunologic response, and quality of life (QoL).

Three cohorts of 3–6 patients each were planned to receive escalating doses of eRapa. Cohort size of 3–6 patients was deemed sufficient to assess the primary objective of safety and tolerability, and was consistent with prior 3+3 dose escalation studies (29). After all patients in a single cohort completed 4 weeks of treatment, a comprehensive safety review was performed to include analysis of all adverse events (AE). If no dose-limiting toxicities (DLT; defined as any AE > grade 2 in severity) occurred, the next cohort was enrolled. If any DLT occurred, an additional 3 patients were enrolled at the same dose. If a second DLT occurred, dose escalation was halted and the prior dose was designated the MTD. If no further DLTs occurred, dose escalation was continued. The study also permitted the enrollment of 3 additional patients at the MTD or highest dose to further characterize safety, pharmacokinetics, immunologic assessment, or QoL.

Study populations

Patients ≥18 years of age with biopsy-proven Gleason ≤7 (3+4) prostate cancer already undergoing active surveillance were enrolled. Exclusion criteria included active or uncontrolled infection, an immunosuppressed state, a second non-prostate primary malignancy, liver disease, or if the patient was already taking a medication known to alter rapamycin metabolism or was deemed to be an inappropriate candidate for active surveillance by the treating physician. Patient and cancer-specific data, including age, race, disease, histology, clinical stage, and Gleason score, were all collected.

Study treatment

The planned doses of eRapa for each cohort were as follows: 0.5 mg weekly (cohort 1), 1 mg weekly (cohort 2), and 0.5 mg daily (cohort 3). The medication was administered orally for a total treatment period of 3 months. Patients were then followed for an additional 3 months to document any delayed AEs or effects of medication withdrawal (total study period of 6 months: 3 months on-treatment and 3 months off-treatment). Patients were discontinued from the study for any of the following reasons: disease progression, unacceptable toxicity, severe AEs or AEs that did not respond to clinical management, physician judgment, or patient preference for withdrawal.

Safety

AEs were evaluated using the Common Terminology Criteria for Adverse Events v5. Severity, number, frequency, duration, and relation of AEs to eRapa administration were assessed. Comprehensive metabolic panels and complete blood counts with differentials were obtained at baseline, week 5, and week 13. Two prespecified comprehensive safety checks were performed after weeks 4 and 12 of treatment and included comprehensive analysis of safety laboratories, AEs, and drug trough analysis.

Pharmacokinetics assessment

Three patients from each cohort provided blood samples for pharmacokinetics analysis. For cohorts 1 and 2, single-dose exposure curves were generated by assessing rapamycin levels in whole blood at 0, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 hours after the first dose of eRapa. For cohort 3, rapamycin trough levels were obtained from samples taken prior to daily doses during the 1st week of treatment to assess time to consistent trough level. In addition, for cohort 3, maximum rapamycin exposure curves were calculated using samples taken at 0, 0.5, 1, 1.5, and 2 hours following a single dose of eRapa during the final week of treatment (week 12). For all cohorts, trough levels were drawn after completing weeks 4 and 12 of dosing for formal safety analysis and to assess long-term pharmacokinetics. For details on the rapamycin assay used, please see Supplementary Data S1.

QoL assessment

Patients were asked to complete the NIH PROMIS-29 Profile v2.1 and PROMIS Cognitive Function v2.0 surveys to assess QoL. Surveys were completed at baseline, 3 months, and 6 months. To score the profile instrument, raw scores were calibrated to standardized T-scores for each category. A standardized T-score of 50 represented the average for the population of the United States (http://www.healthmeasures.net/images/PROMIS/manuals/PROMIS_Adult_Profile_Scoring_Manual.pdf). For negatively worded categories, scores >50 represent scores worse than the U.S. average. For positively worded categories, scores >50 represent scores better than the U.S. average. To score the cognitive function instrument, a raw score was tabulated, with a higher score indicative of "usual" cognitive function and lower scores indicative of diminished cognitive function.

Immunologic assessment

Immune assays were performed prior to treatment and then at 4 weeks, 12 weeks, and 6 months after initiating treatment. T-cell phenotypes were assessed using flow cytometry and categorized into two phenotypes: "naïve/effector" and "exhaustion/inhibition." A full list of immune cell phenotypes studied can be found in Supplementary Table S1. For details on the immunologic assessment assay, please see Supplementary Data S2.

Disease progression

No patients were required to undergo repeat prostate biopsy or PSA. Any repeated PSA or prostate biopsies obtained as part of the standard-of-care active surveillance program were included in the analysis as available. If no additional biopsy data were available, it was assumed that no clinical progression occurred during the study period.

Statistical analysis

Continuous data were summarized and reported as either mean and SD or median and interquartile range. Cohort demographic data were compared using ANOVA, Kruskal–Wallis, or χ^2 tests as appropriate. AEs were stratified by cohort and severity. Frequencies of AEs were compared between cohorts using χ^2 tests. Safety laboratories were compared across timepoints within individual cohorts using a linear mixed model. Pharmacokinetics parameters at individual timepoints were summarized using mean and SE. Total dose exposure was calculated as AUC. QoL scores were compared across timepoints within individual cohorts using a linear mixed model. Immune cell populations were described as mean and SE and compared across timepoints within individual cohorts using a linear mixed model. PSA levels were compared within individual cohorts using a linear mixed model. Patients deemed to be screening failures were excluded from the entire analysis. Data from patients who were dosed, but withdrawn from the study, were included as available. All data analyses were performed using SPSS v24 (IBM).

Results

Cohort enrollment and patient demographics

From August 2018 to June 2019, 15 patients were screened and enrolled. One patient was determined to be a screening failure prior to receiving the first dose of eRapa and was excluded from all subsequent analyses. All the 14 other patients received ≥1 dose of eRapa.

Disposition of patients and demographic data are summarized in **Table 1**. There were 3 patients each in cohorts 1 and 2 and 8 patients in cohort 3. No patients were withdrawn from the study in cohorts 1 or 2. One patient in cohort 3 experienced a grade 3 DLT, prompting 3 more patients to be enrolled. Two of these patients withdrew for non-DLT AEs (grade 1–2) and

Table 1. Demographic data and disposition of patients enrolled by cohort.

Variable	Cohort 1, 0.5 mg weekly ($n = 3$)	Cohort 2, 1 mg weekly ($n = 3$)	Cohort 3, 0.5 mg daily ($n = 8$[a])	*P*
Age (years), mean (SD)	65.4 (8.9)	65.6 (1.8)	68.9 (8.0)	0.71
Race, *n* (%)				
White, non-Hispanic	2 (66.6)	2 (66.6)	5 (62.5)	0.31
Hispanic	1 (33.3)	0 (0.0)	3 (37.5)	
Black	0 (0.0)	1 (33.3)	0 (0.0)	
Other/unknown	0 (0.0)	0 (0.0)	0 (0.0)	
Gleason score, *n* (%)				
6	3 (100.0)	3 (100.0)	6 (75.0)	0.42
7 (3+4)	0 (0.0)	0 (0.0)	2 (25.0)	
Disposition, *n* (%)				
Completed study	3 (100.0)	3 (100.0)	5 (62.5)	
Lost to follow-up	0 (0.0)	0 (0.0)	0 (0.0)	
Withdrawn: disease progression	0 (0.0)	0 (0.0)	0 (0.0)	
Withdrawn: DLT	0 (0.0)	0 (0.0)	1 (12.5)	
Withdrawn: non-DLT AE	0 (0.0)	0 (0.0)	2 (25.0)	

[a]Cohort 3 includes only the 8 patients who received ≥1 dose of eRapa and excludes the screening fail.

were replaced. All patients in cohorts 1 and 2 were Gleason 6, while 2 of 8 (25%) patients in cohort 3 were Gleason 7. There were no significant differences between cohorts in age, race, or baseline Gleason score.

Safety and AEs

A total of 11 of 14 (79%) patients experienced an AE. A single DLT (neutropenia, grade 3) occurred in cohort 3 that resulted in withdrawal from the study. Two non-DLT AEs occurred in cohort 3: papulopustular rash (grade 1) and dizziness (grade 2). Both affected patients were withdrawn. No patient experienced an AE resulting in hospitalization or death. There were 52 AEs across all cohorts: four (7.7%) in cohort 1, one (1.9%) in cohort 2, and 47 (90.4%) in cohort 3. The most common AEs (reported as number of events occurring across all cohorts over total number of AEs) were oral pain (9/52, 17.3%), dizziness (8/52, 15.4%), and fatigue (6/52, 11.5%). The most frequently experienced toxicities (reported as number of patients affected over total number of patients) were fatigue and diarrhea in 4 of 14 (28.6%) patients each. The majority of AEs were grade 1 (36/52, 69.2%). All AEs > grade 1 in severity occurred in cohort 3. There were no statistically significant differences in the distribution of AEs by toxicity grade, the expectedness of the event, or the response to the event when comparing across cohorts (**Table 2**). There were more AEs related to the study medication in cohort 3 relative to the other two cohorts ($P = 0.02$). All toxicities resolved, except for a single grade 1 toxicity (oral pain) in cohort 3.

Hematology and chemistry safety laboratory data were also evaluated. There were no significant differences in any hematology laboratory value measured across timepoints that were concurrently associated with a value outside the normal range. Among chemistry results, glucose levels were significantly lower in cohort 1 at baseline (mean, 83 mg/dL,) and week 5 (mean, 73.3 mg/dL) relative to week 13 (mean, 133 mg/dL; both $P = 0.01$) and were significantly higher in cohort 2 at baseline (mean, 120 mg/dL) relative to week 5 (mean, 65.7 mg/dL; $P = 0.01$). Cohort 3 remained hyperglycemic throughout (means of 102.5, 112.6, and 104 mg/dL at baseline, week 5, and week 13, respectively). These results are summarized in Supplementary Table S2. There were no significant differences in any other chemistry laboratory values measured across timepoints that were concurrently associated with a value outside the normal range.

Pharmacokinetics

Pharmacokinetics measures obtained during the study are reported in **Table 3**. All patients in cohorts 1 and 2 ($n = 3$ each) provided samples at each specified timepoint. In cohort 3, data were available for all patients ($n = 8$) during week 1 (trough level establishment), for 7 patients at week 5, and for 5 patients at weeks 12 (dose response after tough established) and 13. Mean time to maximum serum concentration (C_{max}) was 2.67 (SD, 1.15 hours) and 3.33 hours (SD, 1.15 hours) for cohorts 1 and 2, respectively. C_{max} and AUC were highest in cohort 2. Dose–exposure curves are plotted in **Fig. 1**. The single-dose curve from cohorts 1 and 2 (**Fig. 1A**) demonstrates return to baseline serum levels within 24 hours of receiving a dose. Daily dosing (cohort 3, $n = 8$) produced a stable trough of approximately 2 ng/mL after 48–72 hours in cohort 3 (**Fig. 1B**). The trough measure with highest variability occurred at 24 hours (SE, 0.74 ng/mL). This variability narrowed over subsequent doses (SEs of 0.68 ng/mL at 48 hours, 0.38 ng/mL at 72 hours, 0.25 ng/mL at 96 hours, 0.35 ng/mL at 5 weeks, and 0.50 ng/mL at 15 weeks; **Fig. 1B**). Administration of a single eRapa dose after having already established a stable trough level resulted in a concentration of 4.74 ng/mL (SE, 0.64 ng/mL) at 2 hours (**Fig. 1C**).

QoL

There were no differences in any category measured in cohort 1 over the course of the study. In cohort 2, there was a decrease in anxiety at month 6 relative to baseline (mean T-score, 45.5 vs. 51.8; $P = 0.04$), an increase in fatigue after drug cessation from 3 to 6 months (mean T-score, 40.0 vs. 48.7; $P < 0.01$; **Fig. 2A**), and an increase in participation in activities while on treatment (mean T-score, 50.6 vs. 60.1; $P < 0.01$), followed by a decrease in participation when taken off treatment (mean T-score, 60.1 vs. 52.5; $P = 0.01$). In cohort 3, cognitive function worsened while on treatment, but recovered after cessation of treatment (scores of 129, 120.4, and 128.2 at baseline, 3 months, and 6 months, respectively; **Fig. 2B**) and depression and anxiety symptoms increased from baseline to 3 months (depression mean T-score, 48.7 vs. 55.6; $P = 0.04$ and anxiety mean T-score, 52.4 vs. 58.4; $P = 0.01$; **Fig. 2C** and **D**). Fatigue symptoms increased from baseline to 3 months (mean T-score, 48.4 vs. 54.4; $P = 0.01$), and then remained elevated at 6 months relative to baseline (mean T-score, 48.4 at baseline vs. 56.2 at 6 months; $P = 0.006$; **Fig. 2A**).

Immunologic effects

Immunologic data were available for all patients in cohorts 1 and 2 at each specified timepoint. In cohort 3, samples were collected from all patients ($n = 8$) at baseline, from 7 patients at the 1-month timepoint, and from 5 patients at the 3- and 6-month timepoints. Weekly dosing (cohorts 1 and 2) tended to increase naïve cell populations and reduce central memory cells, while daily dosing (cohort 3) tended to maintain markers of central memory. Representative cell populations that demonstrate these trends are shown in **Fig. 3**. Naïve $CD8^+$ memory T cells (**Fig. 3A**) tended to increase or remain constant during the study in cohorts 1 and 2 (all $P > 0.05$), but significantly increased after cessation of therapy in cohort 3 from month 3 to 6 ($P < 0.001$). Similarly, naïve $CD3^+$ memory T cells (**Fig. 3B**) tended to increase or remain constant during the study in cohorts 1 and 2 (all $P > 0.05$), but increased in cohort 3 during the off-treatment period ($P = 0.04$). $CD8^+$ central memory T cells (**Fig. 3C**) remained constant in cohort 3 while on treatment (all $P > 0.05$), but fell significantly after treatment cessation ($P = 0.03$), and tended to decrease in cohorts 1–2

Table 2. Listing of all AEs and event characteristics by cohort.

	Cohort 1 ($n = 3$)			Cohort 2 ($n = 3$)			Cohort 3 ($n = 8$[a])			
AE	***n***	**%**	**No. of events**	***n***	**%**	**No. of events**	***n***	**%**	**No. of events**	**Total events**
Oral pain	1	33	1	0	0	0	2	25	8	9
Maculopapular rash	1	33	1	0	0	0	0	0	0	1
Pruritus	1	33	1	0	0	0	1	12.5	1	2
Headache	1	33	1	0	0	0	1	12.5	3	4
Diarrhea	0	0	0	1	33	1	3	37.5	3	4
Fatigue	0	0	0	0	0	0	4	50	6	6
Insomnia	0	0	0	0	0	0	2	25	5	5
Constipation	0	0	0	0	0	0	1	12.5	1	1
Bone pain	0	0	0	0	0	0	1	12.5	1	1
Nasal congestion	0	0	0	0	0	0	1	12.5	1	1
Neutrophil count decreased	0	0	0	0	0	0	1	12.5	1	1
Lymphocyte count decreased	0	0	0	0	0	0	1	12.5	1	1
White blood cell count decreased	0	0	0	0	0	0	1	12.5	1	1
Papulopustular rash	0	0	0	0	0	0	1	12.5	1	1
Allergic Reaction	0	0	0	0	0	0	1	12.5	1	1
Dizziness	0	0	0	0	0	0	2	25	8	8
Nystagmus	0	0	0	0	0	0	1	12.5	3	3
Dry mouth	0	0	0	0	0	0	1	12.5	1	1
Laryngeal inflammation	0	0	0	0	0	0	1	12.5	1	1

	Cohort 1 ($n = 3$)			Cohort 2 ($n = 3$)			Cohort 3 ($n = 8$[a])			
Description of AE	***n***	**%**	**No. of events**	***n***	**%**	**No. of events**	***n***	**%**	**No. of events**	***P***
Grade										
Grade 1	3	100	4	1	33.3	1	7	87.5	31	0.65
Grade 2	0	0	0	0	0	0	4	50	15	
Grade 3	0	0	0	0	0	0	1	12.5	1	
Expected										
Yes	2	66.6	3	1	33.3	1	6	75	32	0.77
No	1	33.3	1	0	0	0	3	37.5	15	
Related										
Probable	0	0	0	0	0	0	5	62.5	27	0.02
Possible	2	66.6	2	1	33.3	1	6	75	17	
Unlikely	1	33.3	2	0	0	0	2	25	3	
Response										
Dose not changed	3	100	4	1	33.3	1	4	50	38	0.89
Drug interrupted	0	0	0	0	0	0	1	12.5	2	
Drug withdrawn	0	0	0	0	0	0	3	37.5	7[b]	
Outcome										
Recovered/resolved	2	66.6	2	1	33.3	1	7	87.5	46	<0.001
Recovering/resolving	1	33.3	2	0	0	0	0	0	0	
Not recovered/not resolved	0	0	0	0	0	0	1	12.5	1	
Severe										
Yes	0	0	0	0	0	0	0	0	0	
No	3	100	4	1	33.3	1	7	87.5	47	

[a]Cohort 3 includes only the 8 patients who received ≥1 dose of eRapa and excludes the screening fail.
[b]Three events (1/patient) resulted in withdrawal of study agent. If an AE occurred at the same time as the AE that resulted in study agent withdrawal, it was also coded as "withdrawal."

during the on-treatment period (all $P > 0.05$). In addition, daily dosing appeared to increase levels of exhaustion/inhibition markers, such as $CD4^+$ $LAG3^+$ (**Fig. 3D**), $CD8^+$ $LAG3^+$ (**Fig. 3E**), and $CD8^+$ $PD\text{-}1^+$ (**Fig. 3F**) phenotypes. These inhibitory markers were relatively unchanged in cohorts 1 and 2, but in cohort 3, tended to increase during the treatment period (months 0–3; $CD4^+$ $LAG3^+$, $P = 0.72$; $CD8^+$ $LAG3^+$, $P = 0.03$; and $CD8^+$ $PD\text{-}1^+$, $P = 0.36$), and then substantially increased after treatment withdrawal in cohort 3 (months 3–6; $CD4^+$ $LAG3^+$, $P = 0.002$; $CD8^+$ $LAG3^+$, $P < 0.001$; and $CD8^+$ $PD\text{-}1^+$, $P = 0.003$).

Evidence of clinical disease progression

PSA levels and prostate biopsies were available for all patients at baseline and were only repeated if required per the standard of care. In cohort 1 ($n = 3$), all patients had PSA levels

Table 3. Pharmacokinetics parameters of eRapa.

Description	Variable	Value
Cohorts 1 and 2, 8-hour PK following single dose of eRapa at treatment initiation	C_{max}, ng/mL, mean (SD)	
	0.5 mg weekly	1.24 (0.80)
	1 mg weekly	7.09 (1.85)
	T_{max}, hour, mean (SD)	
	0.5 mg weekly	3.33 (1.15)
	1 mg weekly	2.67 (1.15)
	AUC, ng-hour/mL, mean (SD)	
	0.5 mg weekly	4.89 (4.10)
	1 mg weekly	33.34 (8.09)
Cohort 3, 2-hour PK following a single dose of eRapa after 3 months of daily dosing	C_{max}, ng/mL, mean (SD)	
	0.5 mg daily[a]	2.66 (0.97)
	T_{max}, hour, mean (SD)	
	0.5 mg daily	1.50 (0.61)
	AUC, ng-hour/mL, mean (SD)	
	0.5 mg daily[a]	6.07 (3.07)

Abbreviations: AUC, total dose received; PK, pharmacokinetics.
[a]C_{max} and AUC are reported as values above the baseline concentration.

draw at 3 and 6 months; in cohort 2 ($n = 3$), all patients had levels drawn at 3 months, but none had levels drawn at 6 months; and in cohort 3 ($n = 8$), 3 patients had levels drawn at 3 months and 2 patients had levels drawn at 6 months. There were no significant changes in PSA level in either cohort 1 or 2 across the study. In cohort 3, there was a significant increase in PSA level from baseline to 3 months (from 5.4 to 9.9 ng/mL; $P < 0.01$) and a significant decrease in PSA level from 3 to 6 months (from 9.9 to 0.9 ng/mL; $P = 0.02$; Supplementary Table S3). No biopsies were performed after study initiation in any patient. Therefore, no disease progression was observed from available standard-of-care surveillance data during the study period.

Discussion

In this phase I study of the safety and tolerability of eRapa in patients with Gleason ≤7 (3+4) prostate cancer undergoing active surveillance, we found that eRapa was safe and well-tolerated across all cohorts, with only one grade 3 AE. While the dosing schema of cohort 3 was safe, cohort 3 experienced a higher degree of toxicity relative to cohorts 1 and 2. Cohort 3 also received the highest cumulative dose of eRapa each week. eRapa administration resulted in consistent, dose-dependent C_{max} and AUC following a single dose. In addition, a stable trough developed after approximately 2–3 days of daily dosing. While QoL was not largely affected with weekly dosing, daily dosing did produce transient mild detrimental effects that, except for fatigue symptoms, resolved after cessation of treatment. Immunologic modulation was variable, but an overarching trend toward increased populations of naïve cells on weekly dosing and increased populations of exhaustion cells on cessation of daily dosing emerged. Finally, no patients demonstrated evidence of clinical progression while on treatment based on available data from the patients' active surveillance protocols.

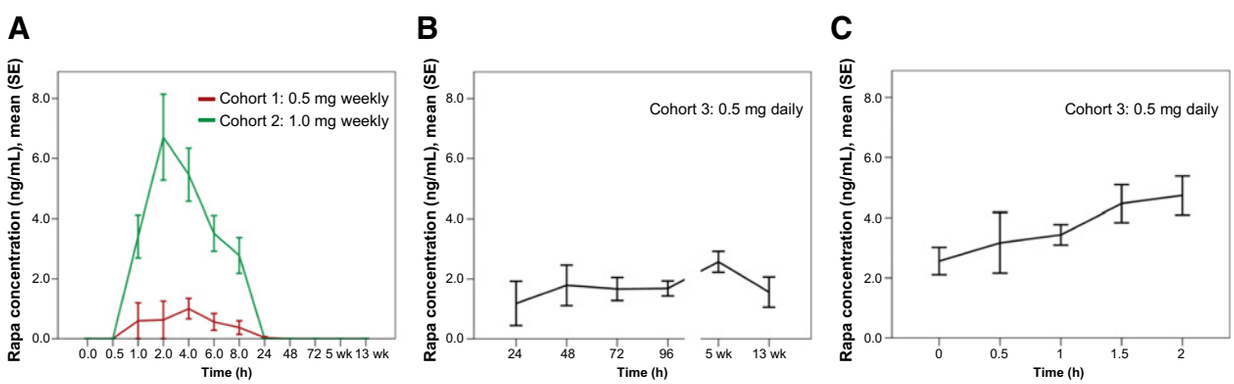

Figure 1.
Pharmacokinetics analysis. **A,** Single-dose pharmacokinetics curves taken from cohorts 1 and 2 at treatment initiation. Serum levels of eRapa return to baseline 24 hours after a single dose. **B,** Time to stable trough level in cohort 3 during week 1 of treatment. Daily dosing produced stable trough levels after 48–72 hours. **C,** Pharmacokinetics measures following a single dose in cohort 3 in the final week of treatment (after establishment of stable trough level). Maximum serum level at 2 hours after the dose was 4.74 ng/mL.

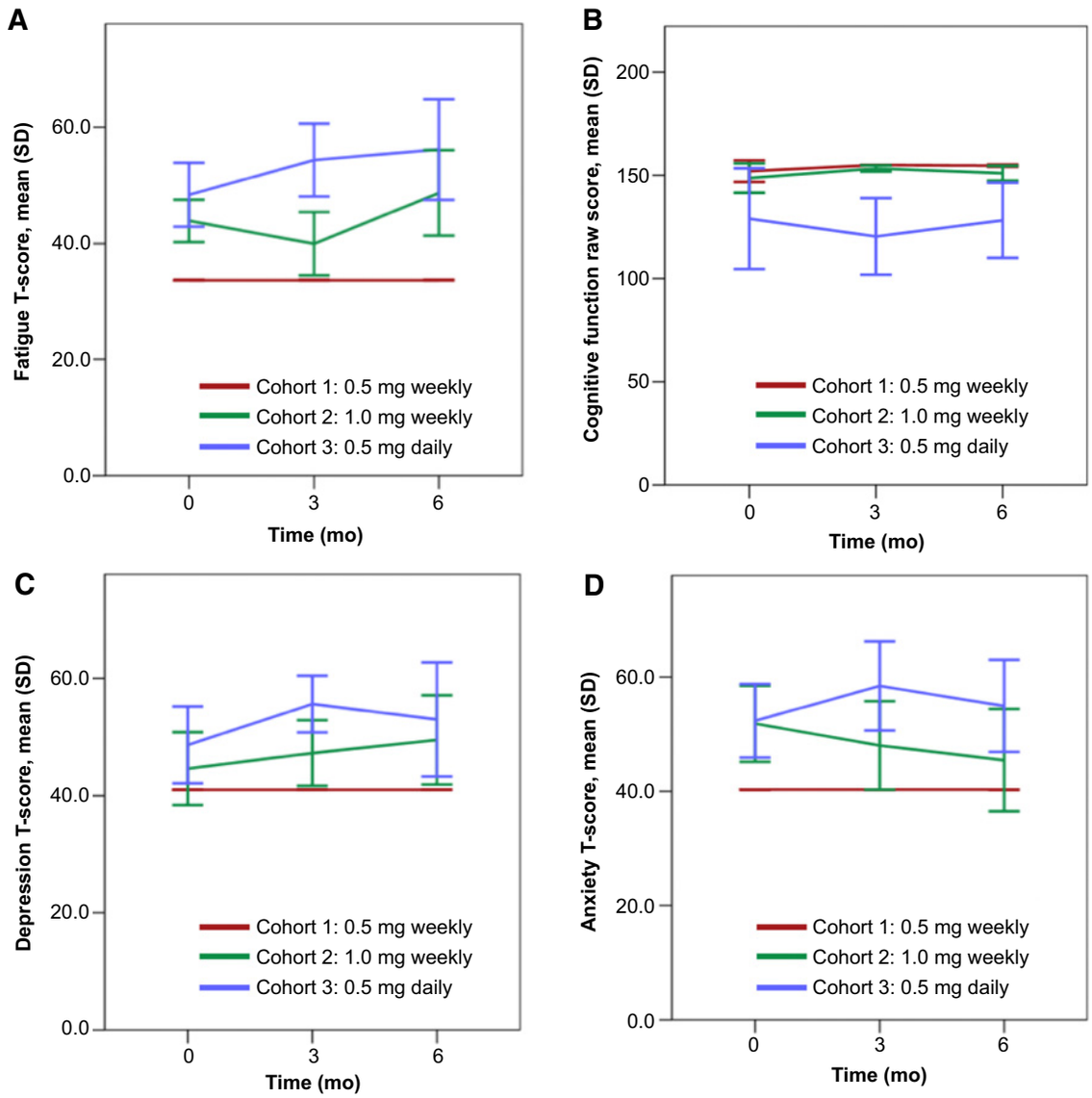

Figure 2.

Cross-cohort assessments of fatigue (**A**), cognitive function (**B**), depression (**C**), and anxiety (**D**). On-treatment: 0–3 months; off-treatment: 3–6 months. In cohort 3, cognitive function decreased and depression, anxiety, and fatigue all increased on treatment; all recovered after cessation of treatment, except fatigue, which remained increased.

Multiple therapies have been investigated to prevent disease progression in patients with prostate cancer (30). In a multicenter, randomized, double-blind, placebo-controlled trial, Fleshner and colleagues randomized 302 men with low-grade (Gleason ≤ 6) disease to receive either dutasteride 0.5 mg daily or placebo for 3 years, with the primary study endpoint of disease progression. At 3 years, 38% of patients in the dutasteride group versus 48% of patients in the placebo group had progressed (HR, 0.62, 95% confidence interval, 0.43–0.89; $P = 0.009$; ref. 31). This study had several important limitations, including the potential interaction of PSA changes caused by dutasteride therapy and the primary outcome of disease progression, as well as a lack of blinding among patients to their PSA values that might have influenced care decisions. Other medications currently under investigation in ongoing trials include enzalutamide (an androgen receptor antagonist; ref. 32; NCT02799745), metformin (through modulation of a number of intracellular pathways; ref. 33; NCT01864096), sipuleucel-T (a personalized cancer vaccine; ref. 34; NCT03686683), and ProstAtak (aglatimagene besadenovec, an oncolytic agent; ref. 35; NCT02768363). Dutasteride and finasteride have also been studied as

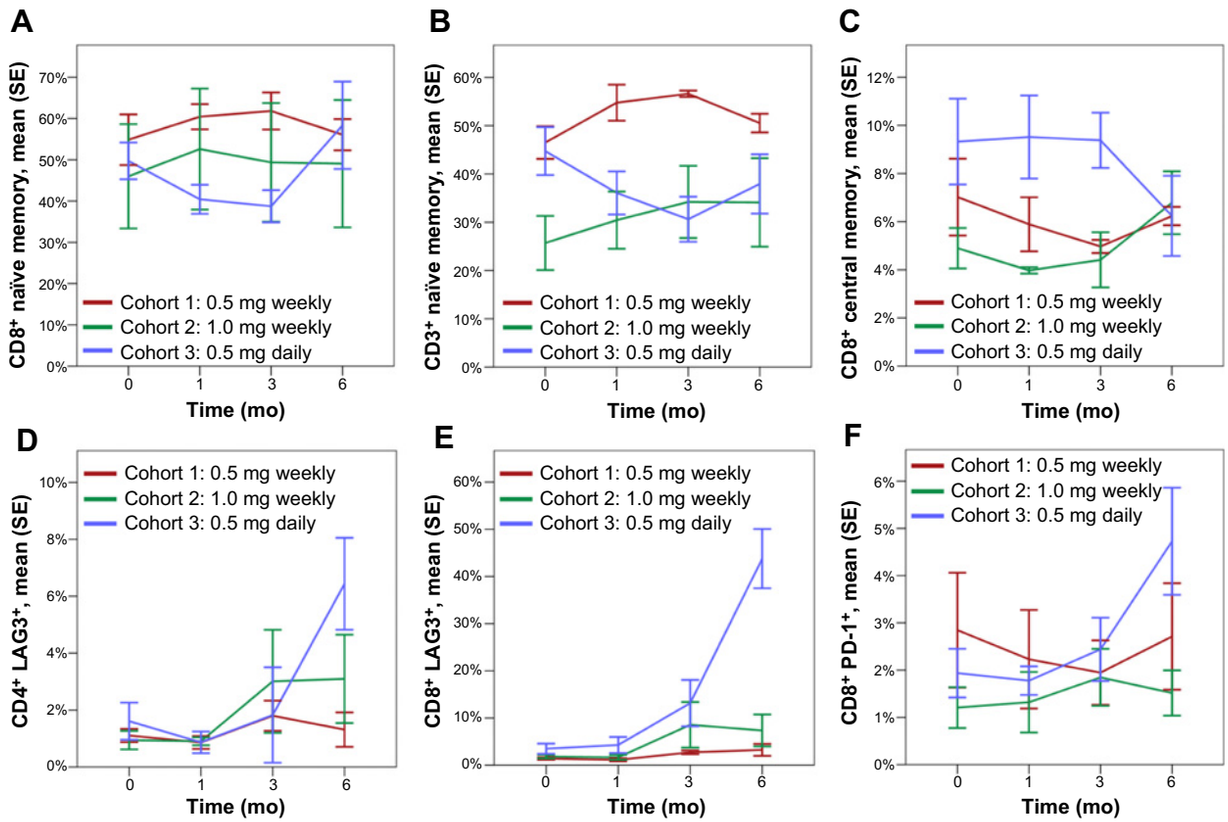

Figure 3.

Immune response to eRapa. Memory/effector immunologic response illustrated by $CD8^+$ naïve memory T cells (**A**), $CD3^+$ naïve memory T cells (**B**), and $CD8^+$ central memory T cells (**C**). Exhaustion immunologic response illustrated by $CD4^+$ $LAG3^+$ T cells (**D**), $CD8^+$ $LAG3^+$ T cells (**E**), and $CD8^+$ $PD\text{-}1^+$ T cells (**F**). In cohorts 1 and 2, there was a trend toward increased naïve memory cells (**A** and **B**) and a decrease in central memory cells (**C**) while on treatment (months 0–3), while in cohort 3, naïve cells (**A** and **B**) significantly increased and central memory cells significantly decrease (**C**) after cessation of therapy. In cohort 3, exhaustion markers (**D–F**) all increased significantly after withdrawal of therapy (months 3–6).

prevention agents in patients with no prior diagnosis of prostate cancer (36). However, a significantly higher proportion of high-grade tumors (Gleason grade, 7–10) was diagnosed in patients treated with each medication (37, 38). Given the potential increased risk for higher grade prostate cancer development, neither of these medications are approved to prevent the development of prostate cancer nor to prevent progression of lower grade disease (https://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021319s015lbl.pdf and https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/020180s037lbl.pdf).

Rapamycin is a well-studied and previously utilized agent with potential to prevent prostate cancer progression. Rapamycin inhibits mTOR, a complex involved in regulating cell growth and macromolecule synthesis and storage (16, 39, 40). Saha and colleagues (20) compared progression of prostatic intraepithelial neoplasia in a HiMyc mouse line (expressive of a murine prostate cancer phenotype) and found that treatment with rapamycin decreased adenocarcinoma *in situ* by 41% relative to control. Furthermore, in a study evaluating safety and pharmacodynamics of oral rapamycin in men with intermediate- and high-grade prostate cancer, Armstrong and colleagues (41) found that 3 mg doses given for 14 days prior to surgery were capable of producing physiologically significant concentrations in prostatic tissue without resulting in DLTs. While the authors reported no differences in tumor cell proliferation or tumor grade as a result of this treatment, this result was not unexpected given the limited treatment time of 14 days. Each of these studies suggest a potential therapeutic benefit to rapamycin in patients with prostate cancer.

The impact of rapamycin on tumor cell proliferation is also likely secondary to modulation of the immune system, a process that would require a longer treatment period than 2 weeks in both *in vivo* and clinical studies to reduce immune exhaustion and senescence while stimulating an effector response. Mannick and colleagues evaluated the immune response in patients recently vaccinated for influenza by randomizing patients to receive either placebo or oral rapamycin at a dose of either 0.5 mg daily, 5 mg weekly, or 20 mg weekly. The authors observed a more robust immune response among the rapamycin-treated patients consisting of significantly higher serum anti-influenza antibody titers and a decrease in $PD\text{-}1^+$

T cells (markers of exhaustion; ref. 24). Laberge and colleagues demonstrated in cell culture that the senescent-associated secretory phenotype (SASP, a surrogate for immune exhaustion) was directly dependent on the mTOR pathway and that rapamycin treatment suppressed secretion of proinflammatory cytokines, IL6 and IL8, while also decreasing production of NF-κB, an important intermediary in a positive SASP feedback loop (42). Collectively, these data suggest an immunoregulatory effect of rapamycin that potentiates an antineoplastic effect.

The amount and timing of rapamycin doses are important considerations, as changes to either can affect the immunologic impact. In comparison with daily dosing, intermittent dosing with rapamycin appears to produce a positive immune impact and antitumorigenic effect. Laberge and colleagues demonstrated that a single dose of rapamycin delivered to cultured cells over 24 hours produced cellular effects that were still visible 7 days after treatment, indicating that daily dosing is likely not necessary (42). Anisimov and colleagues administered rapamycin to mice on an intermittent dosing schedule (2 weeks/month) and found that this dosing regimen resulted in increased lifespan and decreased rates of spontaneous tumorigenesis (43). In our study, daily dosing maintained memory populations and led to significantly higher levels of inhibitor/exhaustion markers following treatment withdrawal, while intermittent dosing resulted in a trend toward higher levels of naïve T-cell populations while on treatment with limited expression of inhibitory/exhaustion markers. Thus, our pharmacodynamics data are in line with previous preclinical work suggesting that intermittent dosing may produce a favorable immunologic impact.

Despite the potential of rapamycin as a chemoprevention agent, major limitations of its use over prolonged periods of time include a narrow therapeutic window and a significant toxicity profile. An agent used for chemoprevention in a low-risk scenario such as that described in this study must have a low toxicity profile and not negatively impact QoL. In this study, toxicities encountered were primarily grade 1 in severity, with the weekly dosing cohorts showing lower rates of toxicity. Similarly, in our QoL analysis, weekly dosing was well-tolerated, but daily dosing decreased cognitive function during the on-treatment period that quickly recovered in the off-treatment period and increased fatigue levels after 3 months of dosing that persisted in the off-treatment period. Cumulatively, our current data support the benefits of an intermittent dosing model.

Another major limitation to widespread use of rapamycin is the significant variation in bioavailability and serum rapamycin levels seen after administration of equivalent doses. Everolimus and sirolimus, two rapalogues, have reported intrapatient AUC variability of 27% and 64% and interpatient AUC variability of 31% and 60%, respectively (26, 27, 44). This wide range of serum levels of rapamycin supplied by the same dose of oral medication necessitates therapeutic drug monitoring (28). The eRapa formulation addressed this limitation by encapsulating rapamycin particles in a polymer matrix, protecting the active drug from degradation in the acidic gastric environment.

As this was the first-in-humans trial of eRapa, a major objective of the study was to assess the pharmacokinetics of this therapeutic. The goal of minimizing toxicity while producing a physiologic effect required a low starting dose with controlled dose escalation, as even the 1 mg doses of eRapa has been associated with toxicity (45). While the optimal circulating concentrations of eRapa necessary to generate a clinically measurable response in the treatment of low-grade prostate cancer are unknown, the level of 4.74 ng/mL achieved in this study was consistent with target levels used in the treatment of patients with familial adenomatous polyposis that resulted in demonstrable clinical benefit (46).

Three findings in our pharmacokinetics analysis warrant further discussion. First, prior studies evaluating sirolimus pharmacokinetics have suggested a linear relationship between dose delivered and AUC (47, 48). In our study, a nearly 7-fold increase in AUC was seen after doubling the dose from 0.5 to 1 mg. Second, rapamycin levels appeared to return to baseline at 24 hours after individual doses, despite a rapamycin half-life of approximately 3 days. Third, despite the eRapa formulation, the variability in AUC was relatively high in cohorts 1 and 3 (84% and 51%, respectively) relative to cohort 2 (24%), although variability in trough levels in cohort 3, as assessed by SE, decreased after subsequent doses. These observations are likely secondary to the fact that the doses used in our study were lower than prior studies evaluating sirolimus pharmacokinetics. The variability in AUC was lowest in the cohort that received the highest dose of eRapa (cohort 2), supporting this hypothesis. However, these findings summarily warrant further exploration in future trials with greater numbers of patients in each dosing arm and comparator rapalogues to assess both absorption and serum bioavailability.

Our study is limited by its relatively small sample size and phase I design. While the study was powered to evaluate safety and tolerability, a comprehensive evaluation of the immunologic response to eRapa or the impact of eRapa on disease progression requires a larger study population. Disease progression was not observed, but progression was also not formally assessed as no patients on active surveillance underwent a repeat prostate biopsy during the study period. To more rigorously assess immune impact and disease progression, futures studies of eRapa in patients with low-grade prostate cancer should involve a larger cohort of patients and incorporate a placebo arm, serial PSA measurements, and pre- and poststudy biopsies. Sampling of the same tumor location pre- and postintervention through imaging-directed biopsy would provide the most accurate assessment of the impact of eRapa on the tumor microenvironment and tumor-infiltrating lymphocyte populations. Finally, a formal bioavailability assessment relative to a comparator medication was not included in our study design. While the SE bars seen in our pharmacokinetics analysis were relatively small and suggest the interpatient variability for the eRapa formulation to be limited, future

studies should more formally evaluate this endpoint and involve a comparator medication to demonstrate this benefit. While the limitations described above are expected in a phase I trial, this first-in-humans study has still provided useful data to help design future studies to assess eRapa efficacy.

Conclusions

Each single dose of eRapa evaluated in this study (0.5and 1 mg) was safe. Weekly dosing with either the 0.5 or 1 mg dose was well-tolerated, had few AEs, and produced a potentially favorable immune impact of maintaining low levels of exhausted T cells. The 0.5 mg daily dose, which resulted in the highest cumulative total dose per week of each of the dosing regimens studied, produced a higher degree of mild toxicity and a transient negative impact on QoL. In future trials, we plan to verify immunologic and clinical benefit through intensified intermittent dosing schedules with the goal of identifying the eRapa regimen that is tolerable and produces the most robust immunologic response and clinical benefit.

Authors' Disclosures

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. D. Hale reported other from Cancer Insight outside the submitted work. I.M. Thompson Jr reports personal fees from Rapamycin Holdings Inc during the conduct of the study. G.E. Peoples reported grants and personal fees from Emtora during the conduct of the study. M.A. Liss reported grants from Emtora during the conduct of the study. No other disclosures were reported.

Authors' Contributions

P.M. Kemp Bohan: Formal analysis, writing–original draft. **R.C. Chick:** Formal analysis, writing–review and editing. **A.E. O'Shea:** Writing–review and editing. **T.J. Vreeland:** Formal analysis, supervision, writing–review and editing. **A.T. Hickerson:** Writing–review and editing. **J.L. Cindass:** Writing–review and editing. **D.C. Ensley:** Writing–review and editing. **D. Hale:** Formal analysis, supervision, writing-review and editing. **G.T. Clifton:** Formal analysis, supervision, writing–review and editing. **V.Y. Sohn:** Supervision, writing–review and editing. **I.M. Thompson Jr:** Conceptualization, supervision, writing–review and editing. **G.E. Peoples:** Conceptualization, resources, formal analysis, supervision, investigation, methodology, writing–review and editing. **M.A. Liss:** Conceptualization, formal analysis, supervision, investigation, methodology, writing–review and editing.

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