Phase II study to evaluate the Efficacy and Safety of Sirolimus in subjects with metastatic, mismatch repair deficient solid tumors after immunotherapy.

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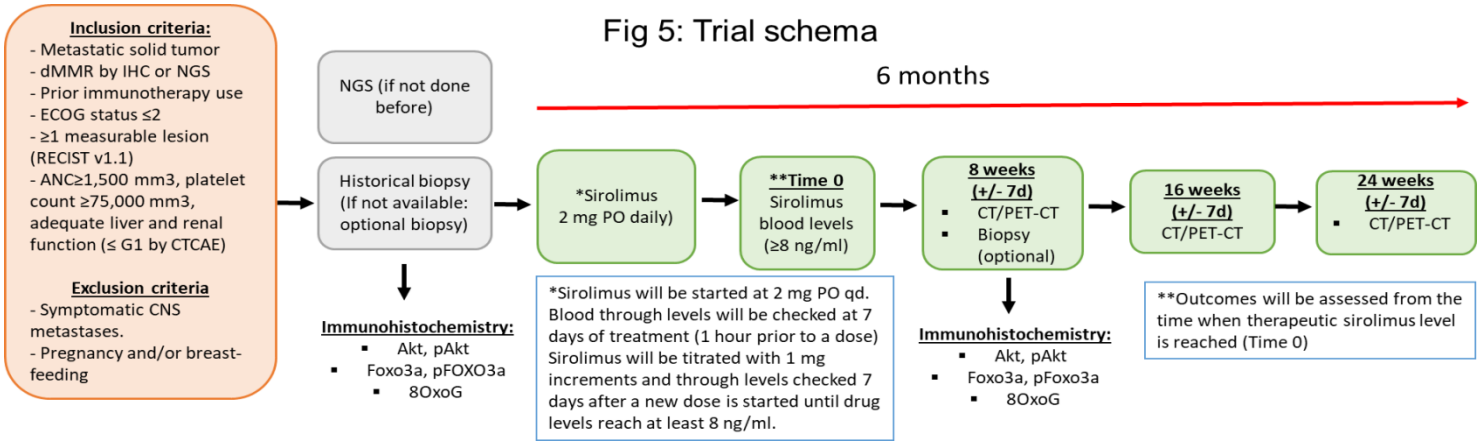
Commercially available agent Sirolimus

TRIAL SCHEMA

"Open-label, Single arm Phase II study of Sirolimus in metastatic, mismatch-repair deficient solid tumors after immunotherapy"

Hypothesis:	- Sirolimus improves ORR by 20% in patients with dMMR solid tumors
Primary Objective:	-To evaluate the efficacy of sirolimus by estimating the ORR as assessed by RECIST 1.1 in dMMR solid tumors
Secondary Objectives:	-To determine other efficacy mesures (PFS, OS) of sirolimus in dMMR solid tumors
	-To assess safety of sirolimus trough evaluation of incidence and severity of adverse events by CTCAE.

Fig 5: Trial schema



Statistical Plan:

Effect size: 20% improvement in ORR (i.e., from 5% to 25%)

Power: 80% **Type 1 error:** 0.05

Expected drop-out rate: 15% **Calculated N:** 19 patients

Contents

1. ABSTRACT

Despite recent therapeutic strategies, including immunotherapy, treatment alternatives for patients with metastatic mismatch-repair deficient (dMMR) solid tumors remain scarce. Pre-clinical data suggests that dMMR tumors are susceptible to rapamycin (sirolimus), a mTOR inhibitor. In these tumors, characterized by higher levels of oxidative stress, sirolimus can exert a cytotoxic effect, led by the failure to repair DNA damage by inhibition of antioxidant enzymes such as FOXO3a triggered by Akt hyperactivation.

This proposal presents a phase 2 clinical trial designed to evaluate the efficacy of sirolimus in patients with dMMR solid tumors after immunotherapy. We hypothesize that sirolimus will increase the overall response rate (ORR) by 20%. To detect such a difference with 80% power and a type-1 error rate of 0.05, 16 participants are required. Accounting for a 15% drop-out rate, we aim to enroll 19 subjects.

After informed consent, adult patients with histologically-proven metastatic solid cancer, dMMR by immunohistochemistry (IHC) or next-generation sequencing, a performance status of ≤ 2 by ECOG, at least one measurable lesion based on Response Evaluation Criteria in Solid Tumors (RECIST v1.1), hemoglobin ≥9 g/dL, absolute neutrophil count ≥1,500 mm^3, platelet count ≥75,000 mm^3, adequate liver and renal function (≤ G1 by CTCAE) will be eligible.

Sirolimus to reach a serum trough level target ≥ 8 ng/ml will be administered orally in 28-day cycles and will be continued until progression of disease evidenced by imaging, unacceptable toxicity or patient withdrawal. Imaging within 21 days of treatment initiation will be considered as baseline. Response to treatment will be assessed by imaging after 8 weeks of achieving therapeutic sirolimus levels, and every 8 weeks thereafter. ORR will be defined as the proportion of patients who achieve complete response or partial response, as assessed by our independent radiology team based on RECIST v1.1. Corresponding exact 95% confidence intervals will also be computed for the true ORR. Side effects will be evaluated and graded using CTCAE at every clinic visit and will be reported as frequencies.

Historical tumor samples will be requested for evaluation of potential biomarkers. In cases where archival samples are not available, an optional biopsy prior to treatment will be offered. All cases with available baseline biopsy, will be offered a repeated biopsy 8 weeks after achieving rapamycin serum target level. Expression levels of Akt, pAkt, Foxo3a, pFoxo3a and 8OxoG will be determined by IHC and reported as 1+ (low), 2+ (intermediate) or 3+ (high). An increase in biomarker expression will be

defined as a change from 1+ at baseline to 3+ after treatment for each specific biomarker. Tumor mutational burden and gene mutations will be evaluated by next-generation sequencing in baseline biopsies. Exploratory analysis will be performed to compare biomarkers between patients with and without objective response.

2. INTRODUCTION

2.1 Background

Deficient mismatch repair (dMMR) is a molecular fingerprint that results from the accumulation of mutations at DNA elements named microsatellites which are characterized by short nucleotide-repetitive sequences in the coding or non-coding regions (1). dMMR has been reported in different solid cancer types with an estimated frequency of 2.2% among more than 50 different cancer types; most commonly represented by colorectal and endometrial cancer (2). It can present as hereditary or sporadic (2). In the former, it is driven by a germline mutation in the mismatch repair (MMR) genes which predispose to colorectal cancer (CRC), endometrial cancer and others such as pancreatic, gastric, urinary tract, brain and skin cancer in Lynch syndrome (3). In the latter, dMMR is generated by a biallelic somatic mutation in the MMR genes or by epigenetic changes, which most commonly cause MLH1 promoter hypermethylation. (4) (5).

Independent of cancer type, dMMR tumors showed an important overall response rate (ORR) with an extended duration of response to programmed-death ligand 1 (PDL1) inhibitors such as pembrolizumab (6). Prior studies have reported ORRs ranging between 31-47.9% and durable responses of more than 6 months in two-thirds of responders. Consequently, pembrolizumab was approved in 2017 by the United States Food and Drug Administration (USFDA) for adult and pediatric patients with unresectable or metastatic dMMR solid tumors that had exhausted other therapeutic options (7). However, treatment options for patients with dMMR solid cancer after immunotherapy, due to progression of disease or inability to tolerate treatment for immune-related adverse events, are limited and represent the focus of current pre-clinical and clinical studies.

2.2 mTOR signaling pathway in cancer

mTOR is a serine/threonine kinase that belongs to the phosphoinositide 3-kinase related protein kinase (PIKK) family and has two different complexes named mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2). mTOR signaling regulates cell growth, metabolism, proliferation and survival through activation of transcriptional factors and inhibition of autophagy. It has been reported that this pathway is overactivated in more than 70% of cancers (8). Consequently, mTOR inhibitors have been developed with currently 3 different FDA-approved drugs in the market: sirolimus, temsirolimus and everolimus. This phase 2 trial will study the prototype mTOR inhibitor, sirolimus, which is a direct mTORC1 inhibitor.

mTORC1 has important components such as the associated protein regulator named RAPTOR and the negative regulator called PRAS40. mTORC1 pathway can be activated via the PI3K/AKt pathway or the ras-MAPK pathway as a response to different stimuli including oncogenic growth factors, insulin, nutrients and hypoxia. In the former, Akt induces phosphorylation that leads to dissociation and inactivation of the TSC2/TSC1 complex that negatively regulates RHEB, an mTORC1 activator. Additionaly, Akt can directly phosphorylate and inactivate PRAS40, which releases RAPTOR that activates mTORC1. In the latter, the ras-MAPK pathway causes activation of MEK 1/2, ERK 1/2 and RSK which can phosphorylate TSC2 causing its dissociation from TSC1 leading to mTORC1 activation (8).

The overactivation of mTOR signaling in cancer follows three different mechanisms: 1) mutations in the mTOR gene; 2) mutations in the components of mTORC1 and mTORC2 or 3) Mutations in suppressor genes or oncogenes. In this regard, PTEN is a tumor suppressor gene that blocks the PI3K pathway. Mutations of PTEN are commonly reported in different solid tumors and are frequently mutated in dMMR tumors (8).

2.3 Pre-clinical Data

2.3.1 In-vitro data

Vilar et al. analyzed 5 different gene expression signatures in CRC aiming to compare the gene expression profile between dMMR and pMMR CRC tumors. This information was used to create an intersection signature, which included genes that were differentially expressed in all the signatures, and a union signature, which included genes that were differentially expressed in any signature. A connectivity map was used to select candidate compounds from 164 tested compounds which allowed the

recognition of 2 drugs (rapamycin and a phosphoinositide 3-kinase named LY-294002) that were selected for testing in-vitro using 8 CRC cell lines. Of these, 2 were pMMR (HT-29 and SW-480) and 6 were dMMR (HCT-116 and Lovo that had MLH1 and MSH2 mutations; HCT-15 and HCT-8 that had MSH6 mutation; SW-48 and RKO that displayed hypermethylation in the promoter of MLH1 gene).

At rapamycin concentrations ranging from 0.6 to 17 uM, **5 of 6 dMMR CRC cell lines were sensitive to mTOR inhibition compared to MSS cell lines with a 6x difference in IC50** (p=0.002). Of note, cell lines with mutation in the MMR proteins MSH2 and MSH6 were the most sensitive to mTOR inhibition. These results were confirmed in proliferation data and highlighted the potential relevance of mTOR inhibition as a targeted therapy among dMMR CRC tumors (Figure 1).

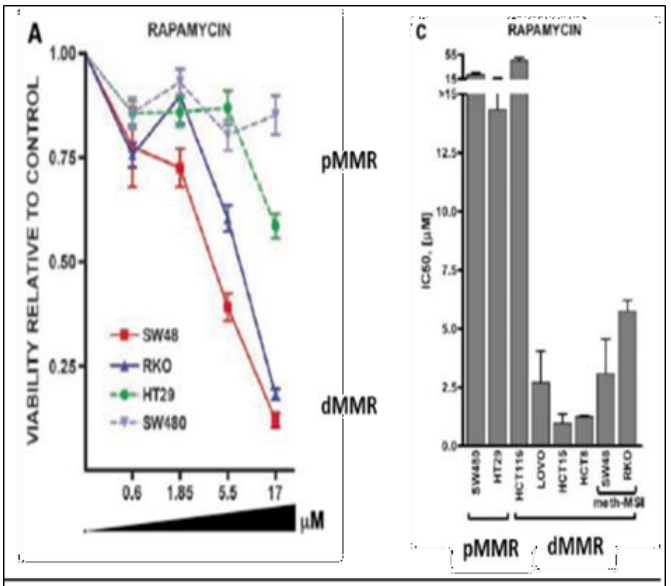


Figure 1: A) Differential sensitivity of colorectal cancer cell lines to treatment with rapamycin for 5 days. B) dMMR confers sensitivity to rapamycin.

2.3.2 Animal model data

Preclinical genetic dMMR mouse models and dMMR patient-derived xenografts were developed in the laboratory of Dr. Edelmann in Einstein. In order to generate mice with tissue-specific inactivation of MSH2 called VCMsh2^{loxP}, Cre-LoxP recombination was used to target MSH2 loss to the intestinal mucosa of Msh2^{loxP} mice using a Villin-cre transgene. Patient tumor cells were injected subcutaneously into the right flank side. All VCMsh2^{loxP} mice spontaneously developed intestinal adenocarcinomas 6-8 months after the inoculation and died 4-6 weeks after diagnosis in the absence of treatment. Rapamycin was injected subcutaneously 3-5 times per week into tumor-

bearing VCMsh2^{loxP} mice. Treatment response was assessed by micro PET-CT at 14 days of treatment and tumor volume measured using the 6"/150 mm Vernier caliper W/fin adj. (Geneva Gage, INC)

In the absence of treatment, dMMR xenografts and pMMR mice evidenced increases in tumor volume and in mean standardized uptake values measured by micro PET-CT at day 14. When rapamycin was administered, all treated tumors (n>100) showed complete regression after 2-3 weeks of treatment and no tumor re-growth was observed in dMMR xenografts (Figure 2); while the tumor continued to increase in pMMR mice (Figure 3 and 4).

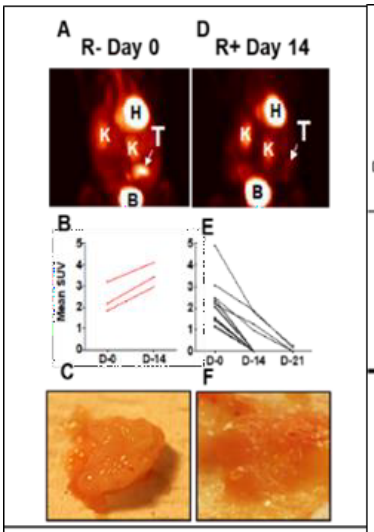


Fig 2: Differences in colonic tumors at day 0 and day 14 with rapamycin (D, E and F) and without rapamycin (A, B and C) in VCMsh2 mice. Legend: (T, tumor; H, heart; K, kidney; B, bladder).

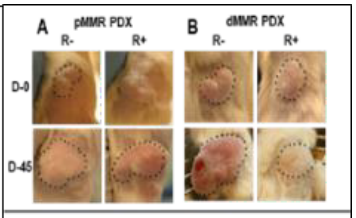


Fig 3: A) Colonic tumor at D0 and D45 after treatment to rapamycin (R+) or D45 without treatment (R-) in pMMR PDX (C1151), B) Colonic tumor at D0 and D45 after treatment to rapamycin (R+) or D45 without treatment (R-) in dMMR PDX (B8020)

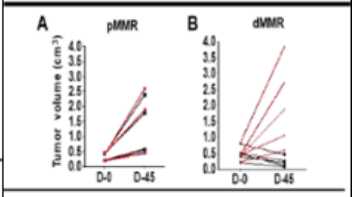


Fig 4: A)Tumor volume in C1151 at D0 and D45 of rapamycin treatment. B) Tumor volume in B8020 at D0 and D45 of rapamycin treatment. Legend: red line: vehicle; black line: rapamycin.

mTOR inhibitors, including rapamycin and analogs, have a well-established cytostatic effect. In addition, a **cytotoxic effect can be exerted in dMMR xenografts as a consequence of higher levels of oxidative stress** generated by Akt hyper-activation and failure to repair the resulting oxidative DNA damage in dMMR tumor cells. mTOR inhibition by rapamycin leads to Akt activation by phosphorylation to pAkt. FOXO transcription factors, including FOXO3a, promote resistance to oxidative stress by induction of antioxidant enzymes. pAkt induces phosphorylation of FOXO3a leading to its inhibition. (Figure 5, A and B). This leads to accumulation of ROS levels

as evidenced by 8-oxoG a common DNA lesion resulting from ROS leading to autophagy and cell death as evidenced by Beclin-1 expression (Figure 5 C).

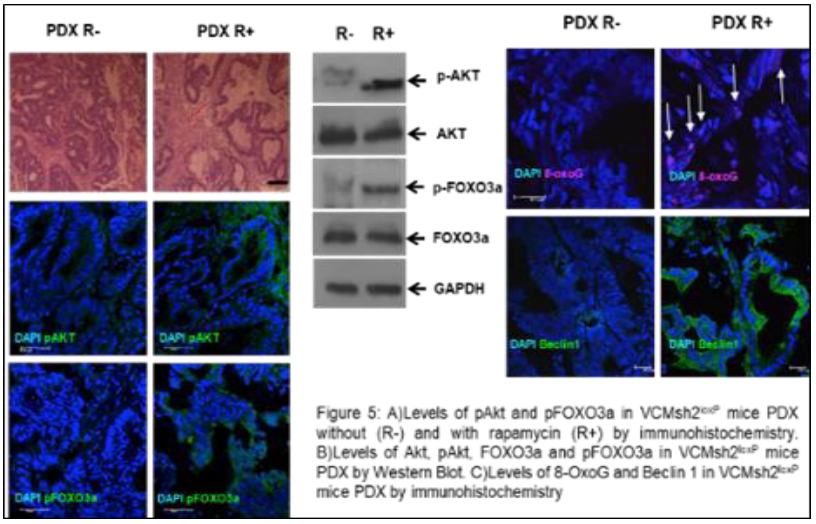


Figure 5: A)Levels of pAkt and pFOXO3a in VCMsh2$^{ex/P}$ mice PDX without (R-) and with rapamycin (R+) by immunohistochemistry. B)Levels of Akt, pAkt, FOXO3a and pFOXO3a in VCMsh2$^{ex/P}$ mice PDX by Western Blot. C)Levels of 8-OxoG and Beclin 1 in VCMsh2$^{ex/P}$ mice PDX by immunohistochemistry

To show that cell death resulting from inability to repair oxidative DNA damage was restricted to dMMR cell lines; we performed experiments with two cell lines: HT29 (pMMR) and LOVO (dMMRI). These showed that pAkt and pFOXO3a and ROS levels increase in dMMR and pMMR cell lines treated with rapamycin; but, 8-oxoG levels increase only in dMMR cell lines. To show the role of pAkt in cell death; experiments adding an Akt inhibitor (Akti) and N-acetylcysteine (an antioxidant) were perfomed, showing that 8oxoG levels do not increase when dMMR cell lines are treated with concomitant rapamycin and Akti or with rapamycin and NAC (Figure 6D). Cell viability is reduced in dMMR cell lines treated with rapamycin; however, the cytotoxic effect is attenuated when Akti or NAC are added (Figure 6E).

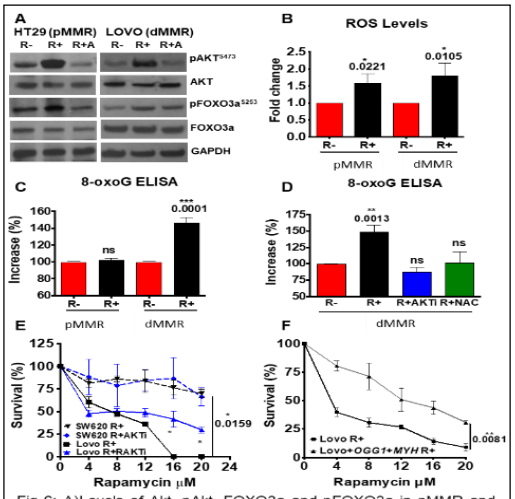


Fig 6: A)Levels of Akt, pAkt, FOXO3a and pFOXO3a in pMMR and dMMR cell lines under different treatments. B, C, D) ROS levels and 8-OxoG levels in pMMR and dMMR cell lines under different treatment. E,F) Cell viability in pMMR and dMMR under different treatments. Legends: (R-): untreated; (R+): rapamycin; (R=Akti): rapamycin and Akt inhibitor (R+NAC): rapamycin and N-acetylcysteine

2.3.3 Safety Data

Sirolimus was FDA-approved in 2010 for the prevention of organ rejection in patients receiving kidney transplants after proving superiority to azathioprine in this setting. Additionally, it was FDA-approved in 2015 for the management of lymphangioleiomyomatosis after showing improvement in lung function in a randomized clinical trial.

A pilot study that included 4 patients (gastric cancer:3, and cholangiocarcinoma:1) assessed the safety and efficacy of rapamycin among patients with refractory solid tumors with PIK3CA amplification/mutation. In this study, sirolimus was administered at 1 mg daily in 28-day cycles. Two patients received 2 cycles and one patient each received 1 and 4 cycles, respectively. Dose reductions/modifications were not required, grade 3 or greater were not observed and there were no treatment-related deaths. Three patients had stable disease (SD) after one cycle and one patient had SD after 4 cycles

2.4 Clinical experience with mTOR inhibitors

In the United States, there are three FDA approved mTOR inhibitors. Of these, 2 of them (everolimus and temsirolimus) have been approved in the treatment of cancer and for organ transplantation rejection prophylaxis in kidney recipients; while the other (sirolimus) has been approved for the latter indication. In general, the most common adverse events are rash, asthenia, and mucositis. Common laboratory abnormalities include anemia, hyperglycemia, hyperlipidemia, and hypertriglyceridemia.

Temsirolimus was FDA-approved for the treatment of patients with advanced renal cell carcinoma (RCC) in 2007 after a randomized clinical trial showed superiority of the combination of temsirolimus and interferon versus interferon alone. Everolimus is FDA-approved for the treatment of patients with advanced renal cell carcinoma refractory, neuroendocrine tumors of gastrointestinal, pancreatic or lung origin, renal angiomyolipoma, tuberous sclerosis, subependymal giant cell astrocytoma and postmenopausal women with metastatic, hormone-positive breast cancer in the second-line setting. Sirolimus was FDA-approved in 2010 for the prevention of organ rejection in patients receiving kidney transplants after proving superiority to azathioprine in this setting. Additionally, it was FDA-approved in 2015 for the management of lymphangioleiomyomatosis after showing improvement in lung function in a randomized clinical trial.

A pilot study that included 4 patients (gastric cancer:3 and cholangiocarcinoma:1) assessed the safety and efficacy of rapamycin among patients with refractory solid tumors with PIK3CA amplification/mutation. In this study, sirolimus was administered at 1 mg daily in 28-day cycles. Two patients received 2 cycles and one patient each received 1 and 4 cycles, respectively. Dose reductions/modifications were not required, grade 3 or greater were not observed and there were no treatment-related deaths. Three patients had stable disease (SD) after one cycle and one patient had SD after 4 cycles (9).

2.5 Relevance and Significance

Despite exciting therapeutic improvement in dMMR metastatic solid tumors, effective treatment options after immunotherapy remain scarce. This study is relevant, as it will evaluate a potentially effective drug targeted to this patient population. This clinical trial has bench-to-bedside translational potential that could provide the foundation for an entirely new therapeutic option for patients with dMMR solid tumors that have run out of other options.

Currently, multiple clinical trials are evaluating the efficacy of mTOR inhibitors alone or in combination for the treatment of different cancer types (10-15); however, none of them have selected dMMR tumors. Therefore, this proposal is highly innovative, as it will focus the intervention to a subgroup of patients that is more likely to benefit, as suggested by our pre-clinical data. Furthermore, the evaluation of tissue biomarkers will confirm whether sirolimus can exert a cytotoxic effect (which has never been reported before) limited to dMMR tumors.

3. OBJECTIVES

3.1 Primary objective:

- To evaluate the efficacy of sirolimus by estimating the overall response rate (ORR) as assessed by Response Evaluation Criteria In Solid Tumors versión 1.1 (RECIST v1.1) in patients with metastatic dMMR solid cancer after immunotherapy (either due to disease progression or to inability to tolerate treatment).

3.2 Secondary objectives:

- To assess potential tissue biomarkers (Akt, pAkt, FOXO3a, pFOXO3a, 8OxoG expression by immunohistochemistry, tumor mutational burden/gene mutations by next-generation sequencing), the effect of sirolimus treatment on these biomarkers, and possible correlation between these biomarkers and clinical response.
- To evaluate other clinical end-points such as progression-free survival, response duration and overall survival of sirolimus in patients with metastatic dMMR solid cancer after immunotherapy (either due to disease progression or to inability to tolerate treatment).
- To evaluate tolerability and safety of sirolimus using Common Terminology Criteria for Adverse Events (CTCAE) grading scale.

4. MATERIAL AND METHODS

4.1 Study design

Open-label, single arm, phase II clinical trial among patients with metastatic, dMMR solid tumors refractory/unable to tolerate immunotherapy.

4.2 Patient selection

4.2.1 Inclusion criteria

- Metastatic solid cancer tumor after immunotherapy (either due to progression of disease or inability to tolerate treatment)
- dMMR by immunohistochemistry (IHC) defined as the loss of expression in any of the four major MMR proteins (MLH1, MSH2, MSH6 and PMS2) or by next-generation sequencing (NGS)
- Age older than 18 at the time of informed consent
- Eastern Cooperative Oncology Group performance status of 0-2
- ≥1 measurable lesion based on RECIST, version 1.1 (16)
- Absolute neutrophil count (ANC) ≥1,500 mm3
- Platelet count ≥75,000 mm3
- Hemoglobin ≥ 9 g/dl
- Aspartate aminotransferase (AST) ≤3.0 times the upper normal limit (UNL)
- Alanine aminotransferase (ALT) ≤3.0 times the upper normal limit (UNL)

- Bilirubin ≤1.5 times the UNL
- Serum creatinine ≤1.5 times the UNL

4.2.2 Exclusion criteria

- Received immunotherapy in the prior 21 days.
- Have not recovered from toxicities of prior treatments to at least grade 1.
- Symptomatic central nervous system (CNS) metastases
- Pregnancy or Breast-feeding.

4.3 Treatment plan

Our reason for selecting sirolimus as the choice of mTOR inhibition for this clinical trial is primarily since all the pre-clinical experiments have been performed with it, and it is feasible to obtain within our estimated budget. Sirolimus has been commercially available since 1999, and fortunately, a generic version has been available since January 2019. Although never commercially approved as an anti-cancer agent, there is ample pre-clinical evidence to support such a role, and there are ongoing cancer clinical trials using this agent (17-19). Moreover, as if often the case, drugs do not get tested appropriately in time to meet the rigors of USFDA approval, nor do they have sufficient patent time remaining to justify the costs to be borne by a pharmaceutical corporation. The very robust pre-clinical data generated by us points to the efficacy of mTOR inhibition in dMMR tumors, and is “pathway driven”, rather than “tissue of origin” driven. We therefore propose to test this hypothesis in a tumor agnostic manner, and will enroll all solid tumors. This approach is similar to the tumor agnostic approach used to test pembrolizumab that led to its subsequent USFDA approval (6).

Based on pre-clinical models, IC50 concentrations in cell viability experiments using dMMR cell lines lie between 8-10 uM, as shown in Fig 3E (3) and is our intended target. As described in its package insert (20), at a daily dose of 2 mg, the steady state concentration is between 7.6 (± 3.1) and 15 (± 4.9) ng/ml. Factoring in the molecular weight of sirolimus (914); a dose of 2 mg is adequate to reach our target concentration. Hence, sirolimus will be started at 2 mg daily. Sirolimus dosing will be titrated to meet serum trough levels of ≥8 ng/ml, assayed at 7 days after starting a new dose, by chromatography/nmass spectrometry. Once adequate serum levels are met (≥8 ng/ml), the same dosing will be continued until progression of disease as evidenced by imaging, or unacceptable toxicity.

4.4 Dosing delays/modifications

Each participant will be evaluated in the oncology cancer center by a physician every 2 weeks for the first 2 cycles and on the first day of each subsequent cycle. The evaluation will include history and physical. Laboratory parameters including cell blood count (CBC), basic metabolic panel (BMP) and liver tests will be checked in every encounter to determine dosing delay/modification. Each cycle is of 28 days in duration starting once therapeutic trough sirolimus levels are achieved in blood.

Side effects will be evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Dose delay and modifications will be required for thrombocytopenia (<75K), neutropenia (ANC<1), or any persistent non-hematologic grade 2 or 3 toxicity. Discontinuation of sirolimus will be required for any grade 4 toxicity, or any toxicity requiring treatment interruption for ≥ 1 cycle.

4.5 Response Evaluation Plan

Each participant will be required to have a recent imaging (CT or PET-CT) within 21 days of treatment initiation which will be considered as baseline. In C1D1, the patient will be assessed in the oncology clinic to document baseline symptoms and laboratory exams including CBC, BMP, liver tests will be performed and repeated at every visit.

Imaging (CT or PET-CT) within 21 days of treatment initiation will be considered as baseline. Response to treatment will be assessed by imaging (CT-CAP or PET-CT) after after 8 weeks of achieving therapeutic sirolimus levels, and every 8 weeks thereafter. Images will be reviewed by the radiology team based on RECIST v1.1 criteria (16).

4.6 Biomarker studies

4.6.1 Prior to treatment initiation:

- Historical tumor samples will be requested for all participants for evaluation of potential biomarkers. In cases where archival biopsies are not available, the patient will be offered a biopsy prior to treatment initiation. This will be optional and will not affect enrollment into the clinical trial. These samples will be used to evaluate the baseline expression of Akt, pAkt, FOXO3a, pFOXO3a, 8OxoG expression will be evaluated by immunohistochemistry in the laboratory of Dr. Edelmann.
- We anticipate that most of the patients would have had next-generation sequencing requested before our evaluation, so, the results will be directly requested. In cases where it was not done; it will be ordered. This will be used to

explore potential mutations that could correlate with treatment response.

4.6.2 During treatment:

- Repeated biopsies after 8 weeks of treatment will be offered to all participants that had an evaluable biopsy prior to treatment initiation. This will be optional and will not affect continuation into the clinical trial. Changes in the expression of Akt, pAkt, FOXO3a, pFOXO3a, 8OxoG expression will be evaluated by immunohistochemistry in the laboratory of Dr. Edelmann.

5. STATISTICAL CONSIDERATIONS

5.1 Endpoints

5.1.1 Primary Endpoint

- Objective response rate (ORR) defined as the proportion of patients who achieve a complete response (disappearance of all target tumors) or a partial response (≥30% decrease in the sum of the longest diameters of target tumors) based on RECIST V1.1 (16).

5.1.2 Secondary Endpoints

- Progression free survival (PFS): PFS is defined as the time from treatment initiation to progression of known metastases or new metastatic site, or death from any cause after a timeframe of 24 weeks.
- Response duration (RD): RD is defined as time from documentation of tumor response to disease progression.
- Overall survival (OS): OS is defined as the time from treatment initiation to death from any cause.

5.2 Sample size

This study is designed as a single arm, single-stage phase II trial to test the null hypothesis that Ho: ORR ≤ 0.05 vs H_A: ORR > 0.05. The target sample size of 16 subjects was determined assuming that the true ORR = 25% with sirolimus, i.e. an absolute improvement in ORR of 20%. If the number of responses at the end of the trial is 3 or more out of 16, we will conclude that sirolimus is beneficial for patients with dMMR tumors and further studies of this treatment will be conducted. If the drug is actually not effective (ORR ≤ 0.05), there is a 5% probability of erroneously concluding that it is effective (Type I error rate). If the drug is truly effective (ORR ≥ 0.25), there is at least 80% probability of correctly concluding that it is (power). To account for a 15% drop-out rate, 19 patients will be enrolled. Because of the limited total sample

size, this trial is designed as a single stage study that does not allow for early stopping due to lack of efficacy.

We anticipate that it will be feasible to enroll 19 participants in a 10-month period based on the patient load treated at Montefiore/Einstein Cancer Center and the commitment of collaborators from other cancer centers in New York City (Lincoln Hospital, Jacobi Medical Center, St. Barnabas hospital, Cornell Cancer Center) to refer eligible patients for this trial. This study will be funded by the Montefiore/Einstein Cancer Center

5.3 Analysis Plan

The ORR will be estimated as the proportion of patients with metastatic dMMR solid cancer refractory to immunotherapy who achieved the endpoint of objective response after treatment with sirolimus. Corresponding exact 95% confidence intervals will also be computed for the true ORR. The distribution of progression-free survival and overall survival will be estimated using the Kaplan-Meier method. Greenwood's method will be applied to estimate confidence intervals.

As a secondary aim, we will evaluate other clinical efficacy end-points such as PFS (time from target serum sirolimus levels to progression of known metastases or new metastatic site, or death from any cause), response duration (time from documentation of tumor response to disease progression.) and overall survival (OS, time from target serum sirolimus levels to death from any cause). The distribution of PFS, DR and OS will be estimated using the Kaplan-Meier method. Greenwood's method will be applied to estimate confidence intervals.

Provided the number of patients who achieve objective response is sufficient, exploratory analyses to assess the association between ORR and the expression of tissue biomarkers will be performed. An exact test for trend for ordered contingency tables will be used to compare the frequency of subjects with low, moderate and high biomarker expression between patients with and without objective response; Fisher's exact test will be used to compare the frequency of subjects that had an increase in biomarker expression after treatment between patients with and without objective response. Gene mutations will be reported as frequencies and compared between patients with and without objective response by Fisher's exact test. The two sample Wilcoxon rank sum or T-test will be conducted to compare the tumor mutational burden between patients with and without objective response.

6. ETHICAL CONSIDERATIONS

6.1 Patient Recruitment

This is a single-center clinical trial. Patients will be recruited in the medical oncology clinic at Montefiore Einstein Cancer Center. Eligible patients will be informed about the clinical trial by the Principal Investigator and will be provided a copy of the ICF in the medical oncology clinic on the day of the initial visit. The Principal Investigator will be responsable for ensuring adequate patient's understanding about the voluntariness of participation, visit and laboratory schedule, benefits and risk of the study and will answer all patient's questions in the initial visit and throughout the study period.

6.2 Patient confidentiality

The Principal Investigator affirms and uphold the principle of the patient's right to protection against invasion of privacy. To ensure this, each patient will be assigned a participant subject number at the time of consent which will be used throughout the study. A patient's source data must only be linked to the clinical study database or documentation via this unique identification number.

6.3 Local Regulations

The study fully adheres to the principles outlined in "Guideline for Good Clinical Practice" (GCP) ICH E6 Tripartite Guideline (January 1997) and remain consistent with the most recent version of the Declaration of Helsinki. The investigators will ensure that the conduct of the study complies with the basic principles of GCP as outlined in the current version of 21 CFR, subpart D, Part 312, "Responsibilities of Sponsors and Investigators" Part 50, "Protection of Human Subjects" and Part 56, "Institutional Review Boards."

6.4 Informed Consent

The participation in the study is voluntary. The ICF for this study is in compliance with ICH GCP, local regulatory requirements, and legal requirements .The ICF used in this study, and any changes made during the course of the study, must be prospectively approved by both the IRB before use.

The Principal Investigator must ensure that each study patient is fully informed about the nature and objectives of the study and possible risks associated with participation. The Principal Investigator must obtain written informed consent from each patient before any study-specific activity is performed. A copy of the informed consent form

(ICF) will be provided to the subject. If applicable, the ICF will be provided in a certified translation of the subject's language. Signed ICFs will remain in each subject's study file and will be available for verification at any time.

This study will not request for a waiver of informed consent.

The Principal Investigator will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided
- Ensure each subject provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed Informed Consent Form(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed Informed Consent Form is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the informed consent form that is approved by an IRB/IEC

6.5 Data storage

Data for this clinical trial will be stored in clinical research forms (CRFs) that will be designed by the Principal Investigator. The Principal Investigator has ultimate responsibility for the accuracy, authenticity, and timely collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms.

Source documentation supporting the CRF data must indicate the patient's participation in the study and must document the dates and details of study procedures, AEs, other observations, and patient status.

6.6 Data and Safety Monitoring Plan

The study data including efficacy, major toxicities, and serious adverse events will be monitored by the Einstein DSMB (data and safety monitoring board). This committee is an integral part of the Albert Einstein Cancer Center, and meets and reviews data every month

7. BENEFIT/RISK ASSESSMENT

7.1 Benefits:

Patients will benefit from inclusion to this clinical trial as they will receive treatment with a drug (sirolimus) potentially efffective for their cancer type based on preclinical studies done at Albert Einstein Medical School. Only patients that have progressed or have been unable to tolerate the standard of care for MSI solid tumors (immunotherapy) will be eligible for this trial.

Inclusion into this trial will not provide monetary compensation, remuneration, reimbursement or pro-rating scales for visits. Inclusion into the trial will not incur in additional charges to the patients. The study drug (sirolimus) will be provided without fee to the study patients. All other ancillary studies will be covered by either insurance or the cancer center: Imaging (CT/PET-CT scans) and routine laboratory studies (CBC, BMP, liver tests) at every visit are considered standard of care and will be covered by insurance. Repeated biopsies will be offered to patients (voluntary) and will be covered by Einstein Cancer Center.

7.2 Risks:

This study carries risk of side effects as patients will be receiving a medication. Sirolimus has been FDA-approved to prevent rejection in patients receiving renal transplants and in patients with lymphangioleiomyomatosis. Other drugs from the same class (everolimus and temsirolimus) are used in patients with breast cancer and renal cell carcinoma. To minimize this risk, patients will be monitored by the Investigators for any side effects which will be graded based on CTCAE v5.0. Based on side effects, the Investigators will decide whether it is safe to continue with the medication or if any changes are required.

To prevent the risk of breach of confidentiality, each subject will be provided with a study ID under which all the clinical information will be stored. Only the research team for this clinical trial will have access to identifiers for the purpose of data collection and data verification.

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