#2741. Combination of Rapamycin with Immune Checkpoint Blockade in a Syngeneic Breast Carcinoma Model

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Introduction and Background

Many advances in the treatment of breast cancer have been driven by the development of targeted therapies that inhibit signal transduction pathways, as well as the development of therapies that activate a patient's immune system to unleash antitumor immunity. The choice of an animal model that mimic aspects of human breast cancer is crucial for evaluating new immunotherapeutic combination strategies. An excellent syngeneic model of breast cancer is 4T1. 4T1-luc2 is poorly immunogenic and shares many characteristics with human breast cancer. To understand the potential benefit from combining a targeted therapy with an immune modulator we utilized the orthotopic 4T1-luc2 model. The mTOR pathway plays an important role in metabolism, cell growth and survival. Targeting mTOR has been an active area of oncology drug discovery and clinical development for breast cancer and other malignancies. In addition, clinical success through blockade of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) has resulted in a paradigm shift for drug development within the oncology community. To this end, we designed a series of experiments to evaluate rapamycin, a first generation mTOR inhibitor, in combination with the immune checkpoint inhibitor antibody against CTLA-4.

Materials and Methods

- Female Balb/c (Envigo) mice were implanted orthotopically with 4T1-Luc2-1A4 cells into mammary fat pad. Forty-eight hours prior to tumor cell implant all mice were dehaired using a depilatory cream.
- A preliminary study evaluated the efficacy and immune modulation of single agent rapamycin. This data was used to define the combination therapy regimen (see Table 1).
- Mice were randomized into treatment groups (see Table 1) when the mean tumor volume in each group was ~100 mm^3. Tumor volumes and body weights were measured thrice weekly for the duration of each study. Bioluminescence images (BLI) were acquired of the thoracic area only when the mean control primary tumor burden reached ~500 mm^3, 750 mm^3 and 1200 mm^3. The primary tumors were shielded to allow any metastatic lesions in the thoracic cavity to be visualized.
- All test materials were purchased from commercial vendors.
- Two weeks post the start of treatment, mice from each group in both studies were collected for flow cytometry analysis.
- All treatment plans were optimized and designed to minimize normal tissue toxicity and produce a homogeneous distribution in the target. Dose distribution maps, dose volume histograms and normal tissue interactions were all taken into account during the treatment planning process.

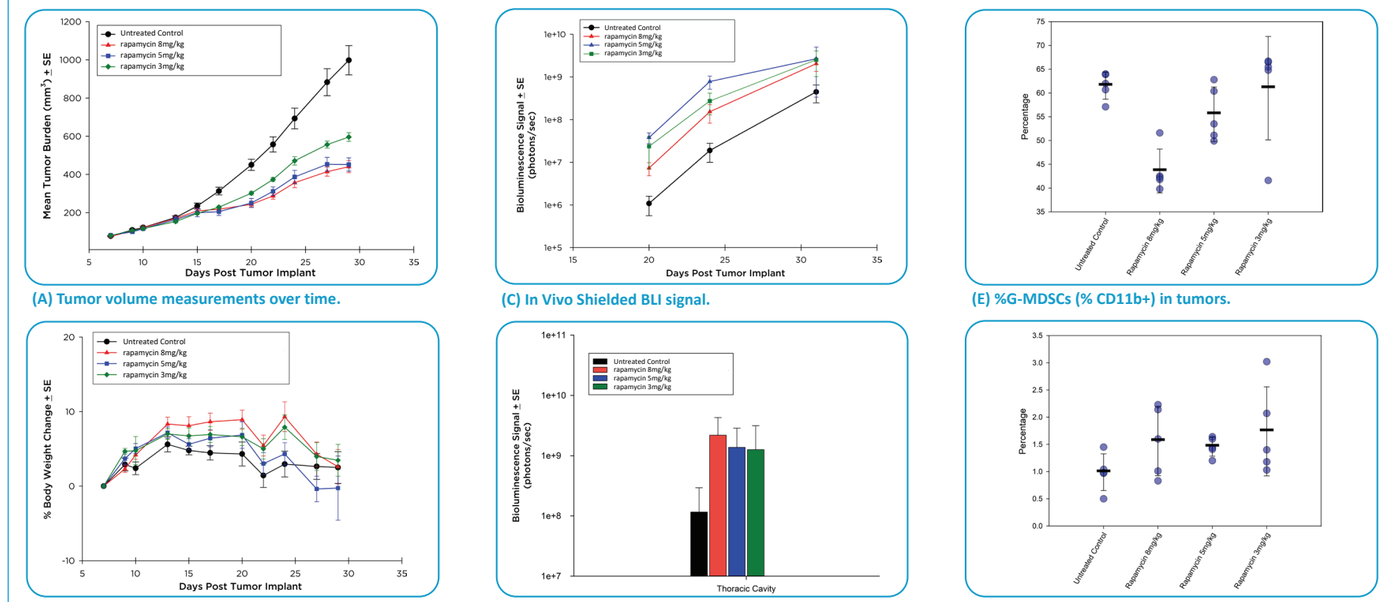

Figure 1. Pilot Anti-tumor Activity of Rapamycin Against 4T1-luc2 Orthotopic Tumor Xenografts.

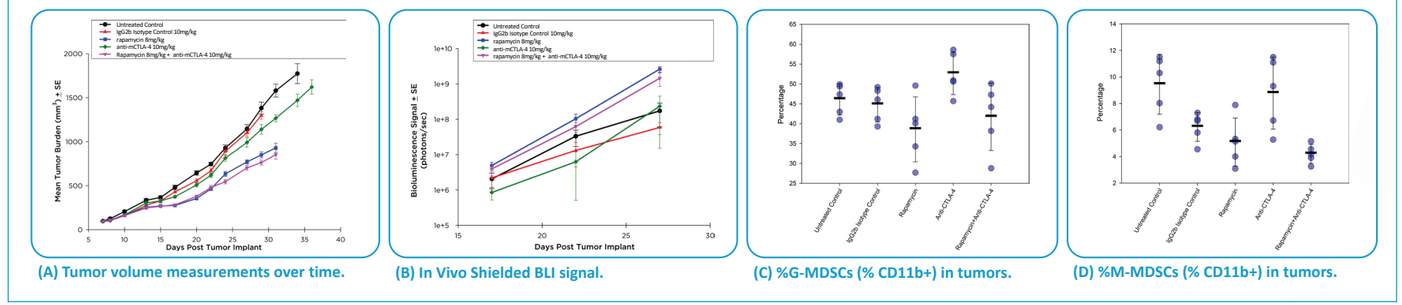

Figure 2. Efficacy Evaluation of Rapamycin Alone And In Combination With Anti-CTLA-4.

Table 1. Basic Design and Data Summary

Group	# of Animals	Drug	Dose (mg/kg)	Schedule	Rx Related Weight Change (%)	Median ΔT/ΔC Day 29 (%)	%G-MDSCs (% CD11b+) in Tumors	%M-MDSCs (% CD11b+) in Tumors
Pilot Anti-tumor Activity of Rapamycin Against 4T1-luc2 Orthotopic Tumor Xenografts								
1	15	Untreated	NA	NA	4.4	NA	61.5	9.7
2	15	rapamycin	8	Q7Dx3	-4.6	42	43.6	11.1
3	15	rapamycin	5	Q7Dx3	-3.9	39	55.5	8.9
4	15	rapamycin	3	Q7Dx3	7.1	56	61	6.5
Efficacy Evaluation of Rapamycin Alone and in Combination with Anti-CTLA-4								
1	15	Untreated	NA	NA	6.9	NA	46.1	9.4
2	15	IgG2b Isotype Control	10	(Q3Dx2; 3 off)x3	8.2	87	44.8	6.2
3	15	rapamycin	8	Q7Dx3	-10.0	58	38.6	5.1
4	15	anti-mCTLA-4	10	(Q3Dx2; 3 off)x3	8.5	78	52.7	8.8
5	15	rapamycin + anti-mCTLA-4	8 + 10	Q7Dx3 + (Q3Dx2; 3 off)x3	-5.1	50	41.7	4.2

Results and Conclusions

- Treatment with rapamycin as a single agent produced modest delay in primary tumor growth. Treatment with anti-mCTLA-4 as a single agent was inactive; consistent with historical data (not shown).
- Single-agent treatment with rapamycin produced a trend in decreasing tumor %G-MDSCs and %M-MDSCs, while single-agent treatment with anti-mCTLA-4 produced a slight trend in increasing the same myeloid cell populations when compared to the isotype controls.
- The addition of anti-mCTLA-4 to the rapamycin treatment did not improve the anti-tumor activity produced by rapamycin alone. However, the combination treatment did produce an overall trend in decreasing both tumor %G-MDSCs and %M-MDSCs population.
- Treatment with rapamycin was able to inhibit primary tumor growth and produce a decrease in the G-MDSC populations, which are associated with T cell impairment and immune suppression. However, the addition of CTLA-4 treatment did not potentiate this shift in the myeloid cell populations, nor did it impact the T cell populations (data not shown). The overall impact of the combination therapy was insignificant.
- Anecdotally, rapamycin treatment alone and in combination was associated with increased lung metastases as determined by in vivo bioluminescence imaging (BLI) when compared to the untreated control. This adverse event is seen in the clinic as well. Patients will often experience increase in metastatic disease following surgical debulking or moderately effective treatment, further supporting the importance of this tumor model (4T1-luc) and its ability to mimic the clinical environment.

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