Galectin-3 inhibition with GR-MD-02 synergizes with agonist anti-OX40 mAb therapy leading to reduced immune suppression and improved overall survival

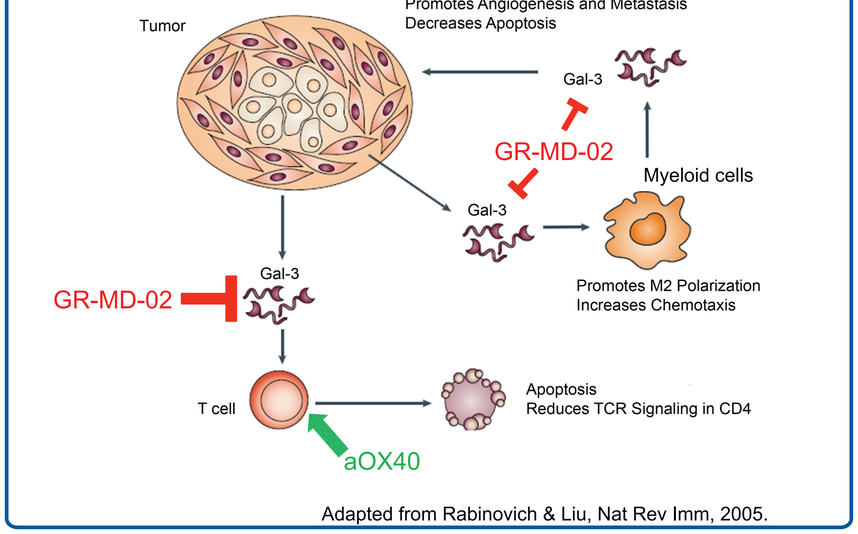
Elizabeth R. Sturgill[1], Stefanie N. Linch[1], Courtney Mick[1], Melissa J. Kasiewicz, Peter G. Traber[2], and William L. Redmond[1]

[1]Robert W. Franz Cancer Research Center, EACRI, Providence Portland Medical Center, Portland, OR 97213 [2]Galectin Therapeutics Inc, 4960 Peachtree Industrial Blvd., Norcross, GA 30071

ABSTRACT

- Galectin-3 (Gal-3) is upregulated on a wide variety of tumors and is associated with poor patient prognosis
- Gal3 recruits myeloid derived suppressor cells (MDSCs) into the tumor microenvironment, thus aiding in tumor escape
- We hypothesized that adding a galectin-3 inhibitor (GR-MD-02) with an agonist anti-OX40 antibody (aOX40) would synergize to promote tumor regression & increased survival by reducing immune suppression
- We report that the combination of GR-MD-02 + aOX40
  - increases survival and decreases tumor burden
  - decreases metastases
  - increases CD8+ proliferation in LN
  - decreases % of Mo-MDSCs & Mo-MDSC expression of PD-L1 & arginase 1 (Arg1) in the tumor
  - Clinical responders to GR-MD-02/pembro therapy have reduced Mo-MDSCs following treatment
- Gal3 inhibition+ aOX40 therapy may reduce myeloid recruitment to the tumor microenvironment, thus reducing immune suppression and subsequently mediating tumor regression and increased survival

BACKGROUND



Adapted from Rabinovich & Liu, Nat Rev Imm, 2005.

RESULTS

Figure 1. Combined GR-MD-02/aOX40 therapy improves survival and reduces metastases

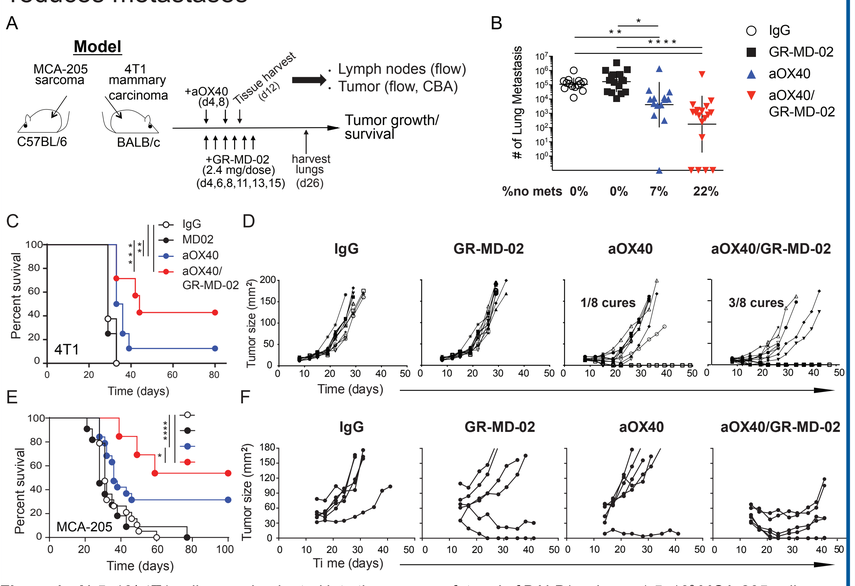


Figure 1. **A)** $5x10^4$ 4T1 cells were implanted into the mammry fat pad of BALB/c mice or $1.5x10^6$ MCA-205 cells were implanted sq in the flank of C57BL/6 mice. Mice were treated with IgG or anti-OX40 (days 4, 8) with and without GR-MD-02 (days 4, 6, 8, 11, 13, 15). B) Lung tissue from mice bearing d26 4T1 tumors were excised and a clonogenic metastasis assay using 6-thioguanine was performed (n=3 independent experiments). C&E) survival and D&F) tumor growth were monitored biweekly and mice were euthanized when tumor area reached 200 mm ^{2}; 4T1 and MCA-205 models, respectively. P<0.05, **P<0.01, ***P<0.001

RESULTS

Figure 2. GR-MD-02/aOX40 increases CD8 T cell proliferation reduces CD4 T cell proliferation, and increases Teff/Treg ratio

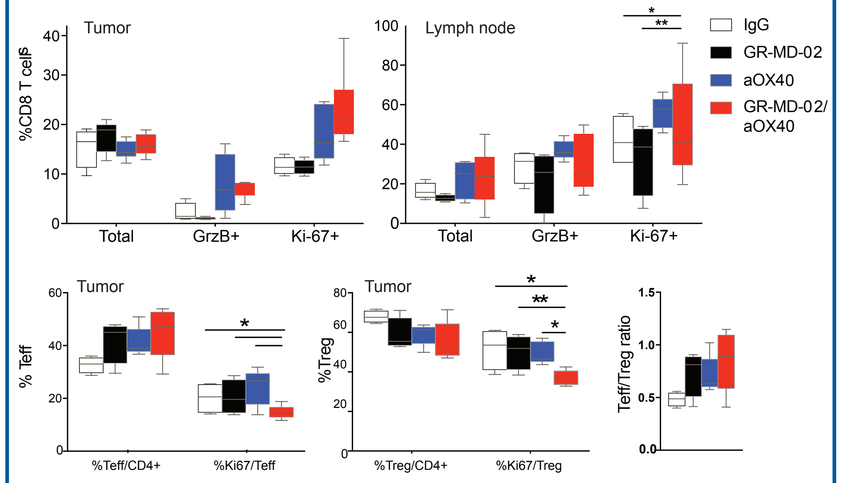


Figure 2. MCA-205 implanation as in Fig 1. Mice were treated with IgG or anti-OX40 (days 10, 14) with or without GR-MD-02 (days 10, 12, 14, 16) ip. Tumors and draining lymph nodes were harvested on day 17 for analysis by flow cytometry. Teff (FoxP3-, CD4+). P<0.05, **P<0.01, ***P<0.001 (ANOVA).

Figure 3. GR-MD-02/aOX40 therapy promotes CTL effector function and survival while decreasing Th2 cytokines

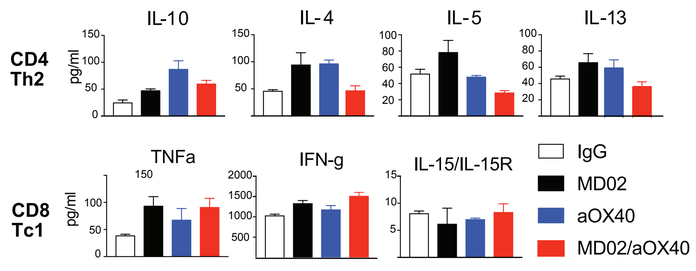


Figure 3. MCA-205 model as in Fig 2. D17, tumors were harvested and pooled (n=4/group). CD4s and CD8s were sorted from tumor then plated on aCD3/aCD28 coated plates.T cells were cultured O/N and supernantants were used for cytokine bead array (CBA). Graphs depict the mean +/-SD of technical replicates for one independent experiment.

Figure 4. GR-MD-02/aOX40 therapy reduces % of Monocytic MDSC within the tumor while modulating expression of Arg1 and iNOS

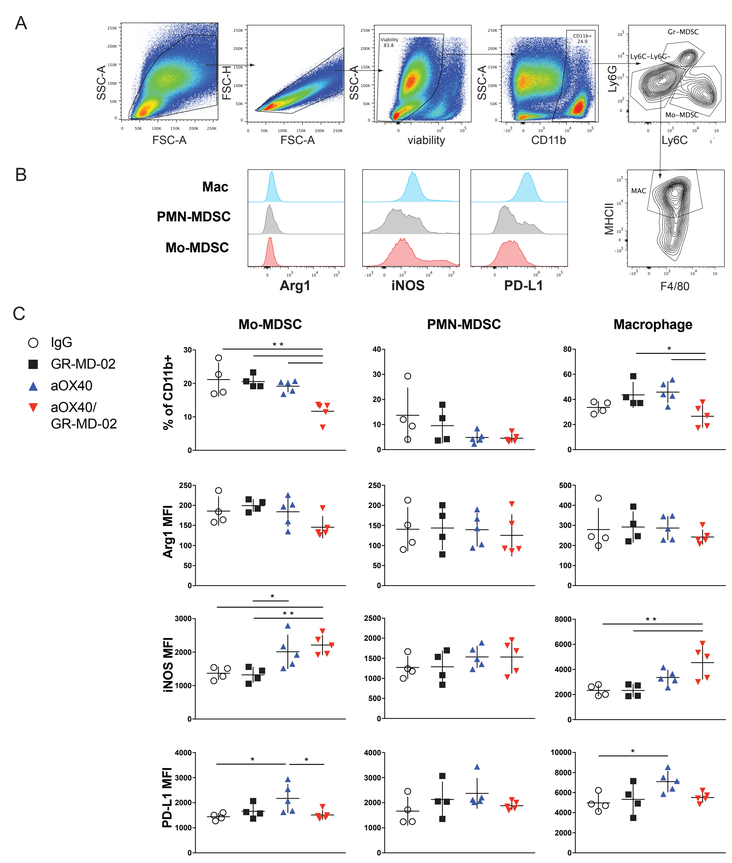


Figure 4. A-C) MCA-205 model as in Fig. 2 A) Gating strategy for Mo-MDSC, PNM-MDSC, and Mac. B) Representative histogram of mean fluroscence intensity (MFI) for Arg1, iNos , and PD-L1 expression in Mo-MDSC, PNM-MDSC, and Mac. C) % of Mo-MDSC, PMN-MDSC, and Mac of CD11b+ cells and MFI for Arg1, iNos , and PD-L1 expression.*P<0.05, **P<0.01, ***P<0.001 (ANOVA).

RESULTS

Figure 5. Responders to GR-MD-02/pembrolizumab therapy have reduced Mo-MDSCs following treatment

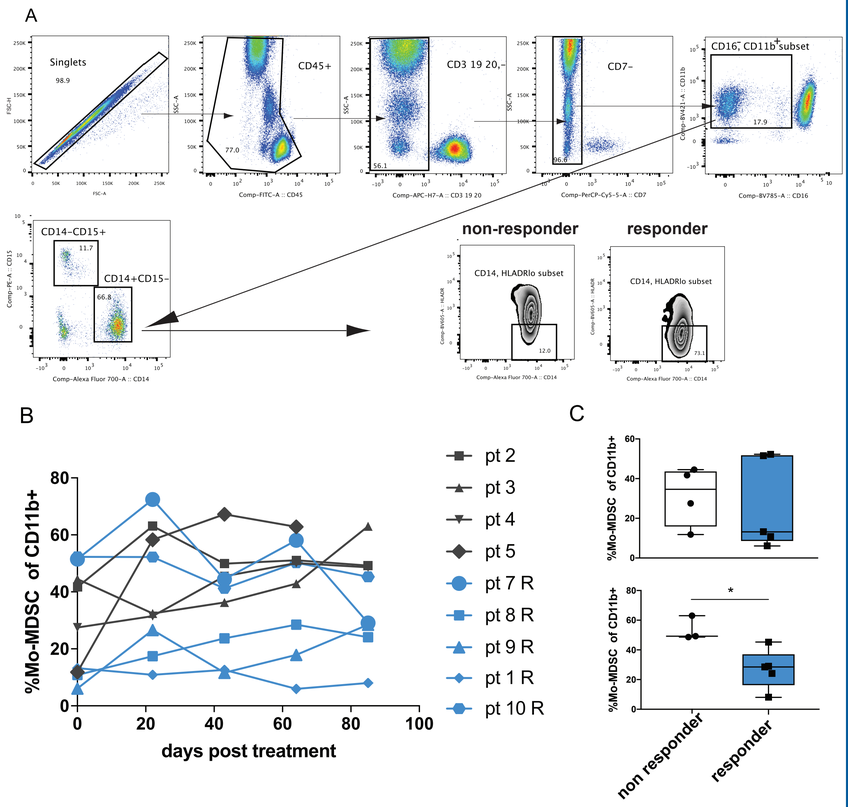


Figure 5. Patients with metastatic melanoma or head and neck squamous cell carcinoma were given GR-MD-02 (2mg/kg or 4 mg/kg, five doses) and pembrolizumab 200 mg (fixed dose) IV every 3 weeks. Respose rates (RECIST 1.1) were determined by CT scan on d85 (NCT02575404). A) Gating strategy for Mo-MDSCs (CD11B+, CD14+, HLA-DR-) B) % of Mo-MDSCs of CD11b+ for each responder (—) and non-responder (—) every 3 wks. C) % of Mo-MDSCs of CD11b+ at BL vs d85. *P<0.05, Student T-test.

CONCLUSIONS

Combination therapy of GR-MD-02/aOX40

- ✓ Increases survival & decreases lung metastases
- ✓ Increases CD8 proliferation in LN
- ✓ Promotes a CTL effector cytokine profile while decreasing Th2 cytokines
- ✓ Reduces Mo-MDSC "-like" cells within the tumors
  - Decreases in Arg1 and PD-L1
- ✓ Clinical responders (see **Poster #P287)** to GR-MD-02/pembro therapy have reduced Mo-MDSC post-treatment

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