

TARGETING VSIG4, A NOVEL MACROPHAGE CHECKPOINT, REPOLARIZES SUPPRESSIVE MACROPHAGES WHICH INDUCES AN INFLAMMATORY RESPONSE IN PRIMARY CELL IN VITRO ASSAYS AND FRESH HUMAN TUMOR CULTURES

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Background VSIG4 (V-set immunoglobulin-domain-containing 4) is a B7 family related protein with known roles as a complement receptor involved in pathogen clearance as well as a negative regulator of T cell activation by an undetermined mechanism.¹⁻³ VSIG4 is expressed in tumor associated macrophages (TAMs) with exquisite specificity. In cancer, increased expression of VSIG4 has been associated with worse survival in multiple indications, including non-small cell lung cancer, multiple myeloma, ovarian cancer, and glioma, suggesting an important role in tumor immune evasion.³⁻⁶ Based upon computational analysis of transcript data across thousands of primary cancer and normal tissue samples, we hypothesized that VSIG4 has an important regulatory role in promoting M2-like immune suppressive macrophages in the tumor microenvironment, and that targeting VSIG4 via a monoclonal antibody could relieve VSIG4-mediated macrophage suppression by repolarizing TAMs to an inflammatory phenotype capable of coordinating an anti-tumor immune response.

Methods The ability of anti-VSIG4 antibodies to repolarize M2-like macrophages and induce T cell activation was assessed in vitro and ex vivo, by measuring production of inflammatory mediators. *In vitro* assays were performed primarily with M-CSF plus IL-10 driven monocyte-derived M2c macrophages from healthy donors. *Ex vivo* assays were performed with fresh, patient-derived tumor samples in culture. To determine whether targeting VSIG4 can lead to an anti-tumor effect *in vivo*, syngeneic mouse models were dosed with anti-mouse VSIG4 antibodies and characterized for changes in tumor volume and immune cell populations.

Results In *in vitro* and *ex vivo* assays anti-VSIG4 antibodies repolarize M2 macrophages and induce an immune response culminating in T cell activation. Targeting VSIG4 upregulates pro-inflammatory cytokines in M2c macrophages, as well as upregulates pro-inflammatory myeloid-derived cytokines and T cell-derived cytokines in M2c macrophages co-cultured with autologous T cells in the presence of staphylococcal enterotoxin B (SEB) activation. To assess targeting VSIG4 in a relevant translational model, fresh, patient-derived tumor samples were treated *ex vivo* with anti-VSIG4. Across multiple tumor types, anti-VSIG4 treatment resulted in a significant upregulation of cytokines involved in TAM repolarization and T cell activation, and chemokines involved in immune cell recruitment, at levels greater than observed by treatment with anti-PD-1 or a clinical macrophage repolarizing agent (anti-ILT-4). *In vivo*, tumor growth inhibition is observed in syngeneic mouse models dosed with anti-mouse-VSIG4 alone and in combination with anti-PD-1.

Conclusions Taken together, these data suggest that VSIG4 represents a promising new target capable of stimulating an anti-cancer response via multiple key immune mechanisms.

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Ethics Approval All legal and ethical requirements were met with regards to the humane treatment of animals described in the study. The animal study was conducted in compliance with CRL IACUC under IACUC No. 1033.

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