

Reprogramming of tumor associated M2 macrophages with antisense oligonucleotide-loaded exosomes results in potent single-agent antitumor activity

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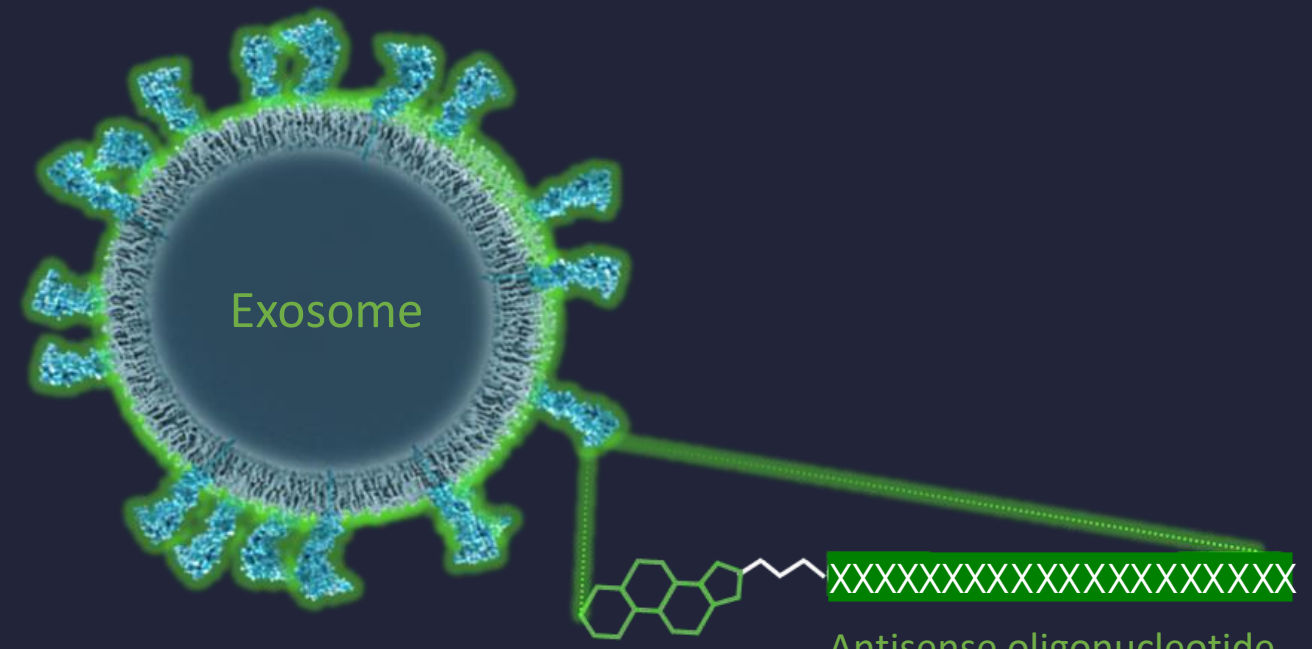


Introduction

- Highly immunosuppressive (M2 phenotype) macrophages are potent divers of tumor growth
- Targeting M2 macrophages with anti-CSF1R therapies show modest single agent activity
- Decreasing the expression of key immunosuppressive transcription factors STAT6 and C/EBP β could effectively reprogram M2 macrophages to a pro-inflammatory M1 phenotype
- We have developed novel, engineered exosomes that selectively deliver antisense oligos (ASOs) to M2 macrophages to selectively silence STAT6 and C/EBP β expression

exoASO, a novel engineered exosome

- Exosomes are natural intercellular messengers that can deliver bioactive payloads
- exoASO is a novel engineered exosome stably loaded with antisense oligonucleotides (ASOs)
- Cholesterol-tagged ASOs targeting STAT6 or C/EBP β are exogenously loaded onto the exosome surface

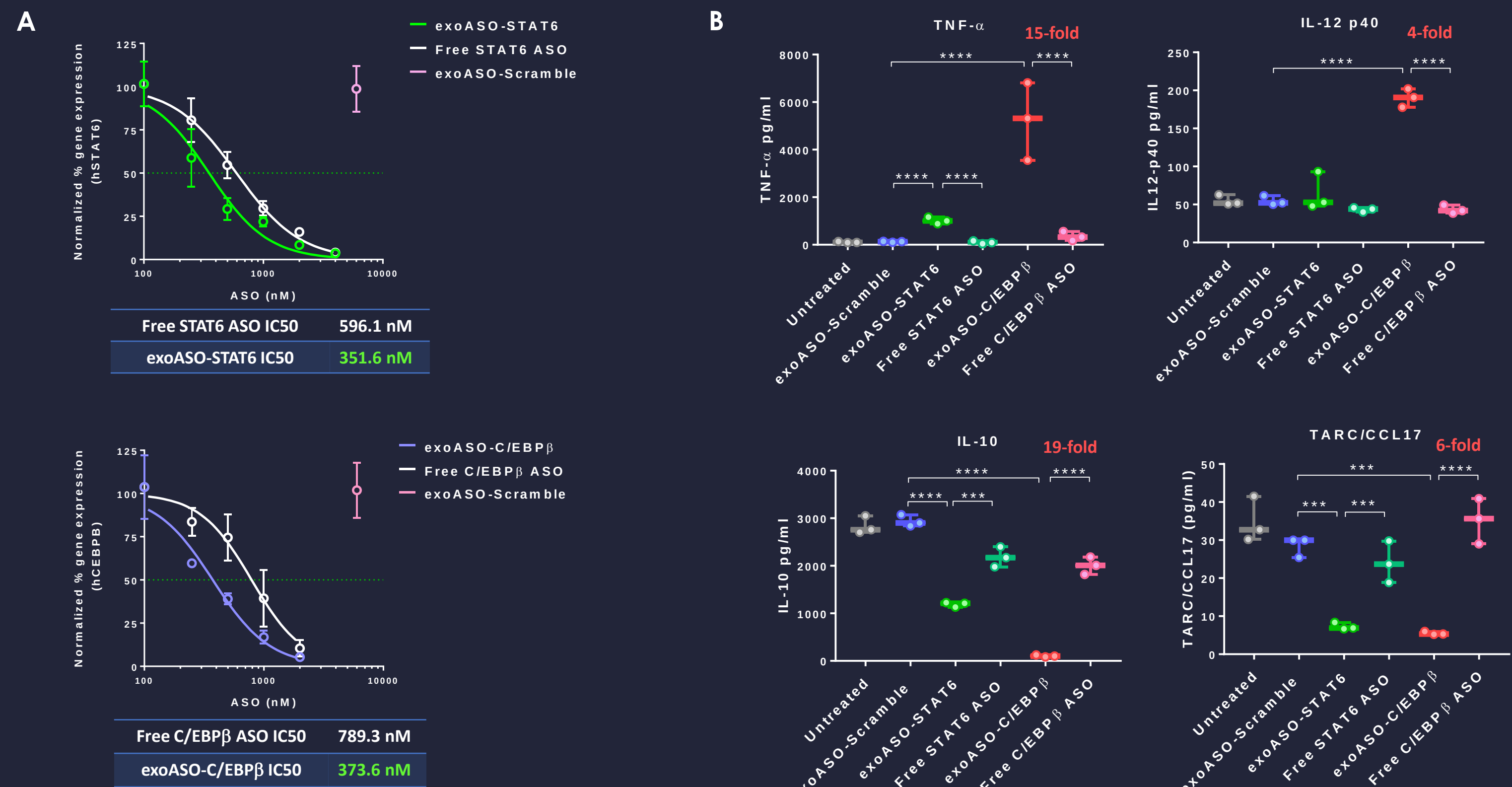


Selective delivery of ASO to tumor associated myeloid cells



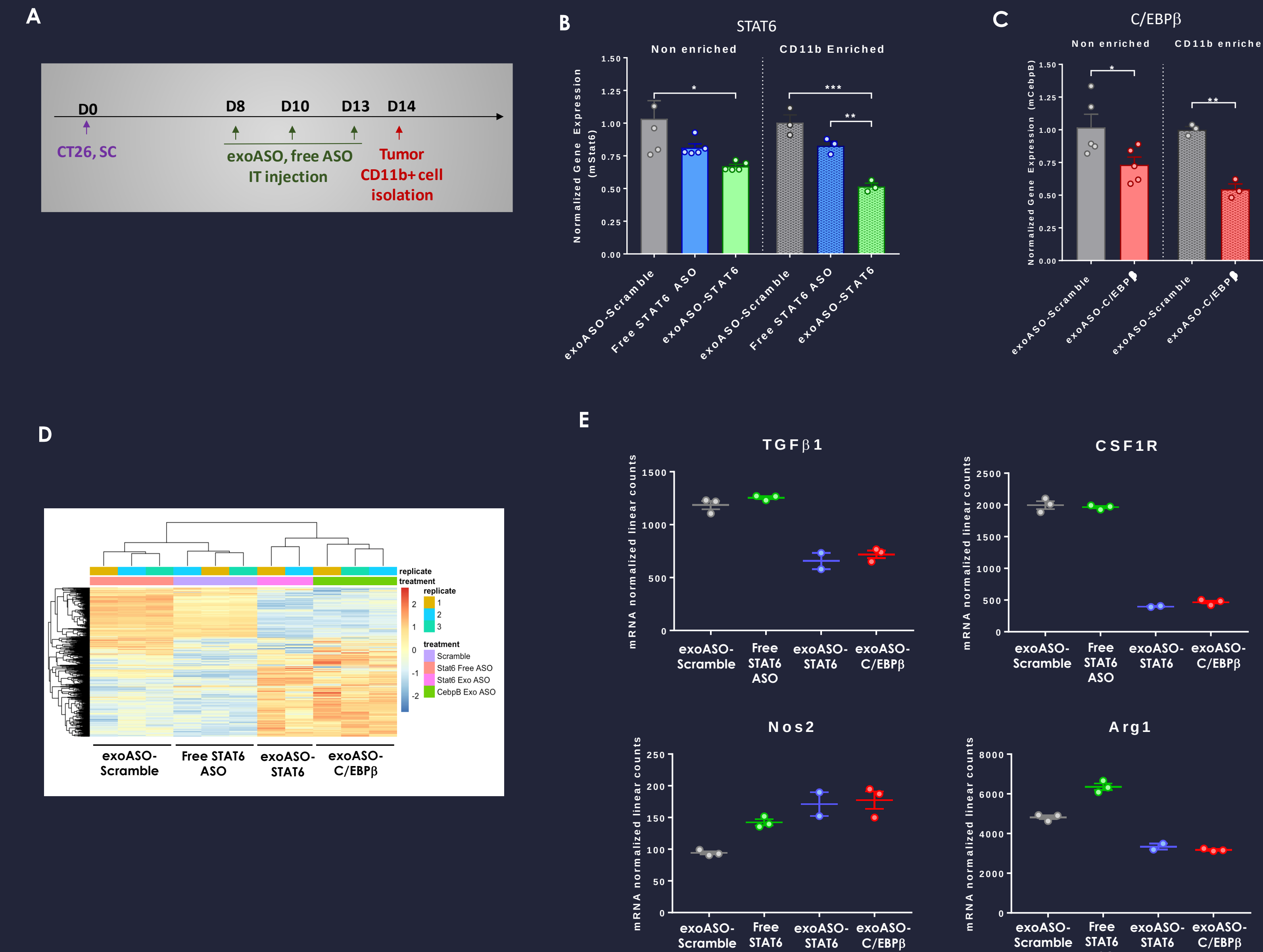
Exosome tropism to myeloid cells promotes selective delivery of ASO. A-B: BALB/c mice bearing CT26 subcutaneous (sc) tumors received one intravenous dose (8 μ g) of fluorescently labeled exoASO or free ASO. One hour later, peripheral blood (C) and tumor (D) were collected and analyzed by flow cytometry. C: CT26 sc tumors received one intratumoral (it) dose (4 μ g) of fluorescently-labeled exoASO. One hour later, tumors were dissected and enzymatically digested, and tumor cell suspensions analyzed by flow cytometry. MDSC=myeloid-derived suppressor cells. mMDSC= monocytic MDSC. gMDSC= granulocytic MDSC. cDC2= type 2 conventional dendritic cells. cDC1= type 1 conventional dendritic cells.

STAT6 and C/EBP β mRNA silencing by exoASO repolarizes human M2 macrophages in vitro



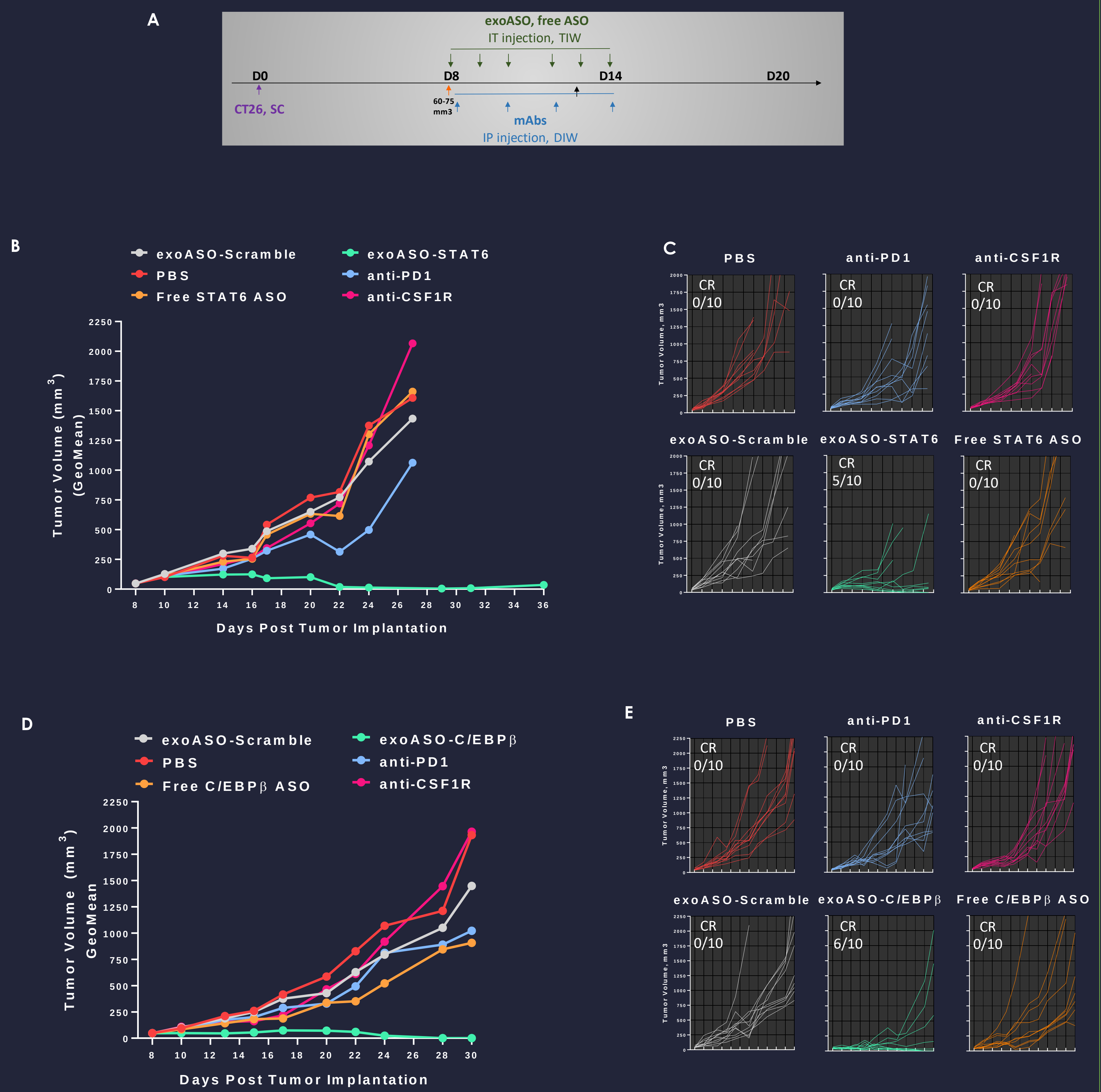
Effective M2 to M1 macrophage reprogramming in vitro by exoASO. A: Human primary M2 macrophages were incubated for 48 hr. with equivalent doses of exoASO and free ASO targeting STAT6 (top) or C/EBP β (bottom). Gene expression levels were analyzed by qPCR. One representative donor of five is shown. B: Primary human M2 macrophages were treated with 4 μ M exoASO or free ASO and cytokine production was analyzed after 24 hr treatment with LPS (10 ng/ml) using a multiplex flow cytometry assay. One representative donor of three is shown. ****, P < 0.0001 and ***, P < 0.001 by one-way ANOVA with Tukey's multiple comparison test, compared to exoASO-scramble or free ASO. exoASO-C/EBP β or exoASO-STAT6 as compared to exoASO-Scramble induced TNF- α by 41-fold and 8-fold respectively. IL-10 levels were decreased by 28-fold and 2.5-fold by exoASO-C/EBP β and exoASO-STAT6, respectively, compared to exo-Scramble.

Effective reprogramming of M2 macrophages in vivo by knockdown of STAT6 and C/EBP β



In vivo knockdown and reprogramming by exoASO. CT26 tumors were treated intratumoral (IT) with 4 μ g of free ASO or exoASO according to schematic in A). After treatment, tumor-associated myeloid cells were isolated using CD11b-positive selection magnetic bead isolation (80% enrichment). A: Dosing scheme. B-C: Target gene KD in pre- and post-enrichment samples were analyzed by qPCR in whole tumor and in tumor CD11b+ cells. D-E: Gene expression analysis in tumor myeloid cells was performed by Nanostring using a mouse myeloid panel. ***, P < 0.001 by two-way ANOVA with Tukey's multiple comparison test, compared to exoASO-scramble or free ASO.

Monotherapy with exoASO-STAT6 or exoASO-C/EBP β results in significant anti-tumor activity



Anti-tumor activity of exoASO-STAT6 and -C/EBP β as single agents. CT26 tumor cells were implanted subcutaneously in the flanks of mice (n = 10 per group). ExoASO and free ASO were dosed intratumorally and antibodies intraperitoneally following dosing regimen in A. A: Dosing scheme. B-C: exoASO-STAT6 or free ASO. D-E: exoASO-C/EBP β or free ASO. Geometric means of tumor volumes are depicted in B and D. Individual tumor growth curves are shown in C and E. CR: Number of complete responses.

Summary

- exoASO is a novel, engineered exosome that can selectively deliver antisense oligonucleotides to M2 macrophages.
- exoASO enables selective silencing of key transcription factors, e.g., those that control the immunosuppressive program, STAT6 and C/EBP β in M2 macrophages.
- Effective silencing of STAT6 and C/EBP β by exoASO in M2 macrophages results in reprogramming to an M1 phenotype, with up to 15-fold induction of proinflammatory cytokines and up to 19-fold down regulation of anti-inflammatory cytokines in vitro.
- exoASO-STAT6 and exoASO-C/EBP β treatment of mice with CT26 tumors resulted in 50-60% complete response as a monotherapy.
- No significant anti-tumor activity was observed with anti-PD-1 or anti-CSF1R monotherapy in this model.
- Collectively, exoASOs against STAT6 and C/EBP β represent a first-in-class strategy to target tumor associated myeloid cells in a highly selective manner.

