MYELOID
Therapeutics, Inc.

In vivo programming of myeloid cells by mRNA mediated delivery of novel Fcα fusion receptor activates anti-tumor immunity

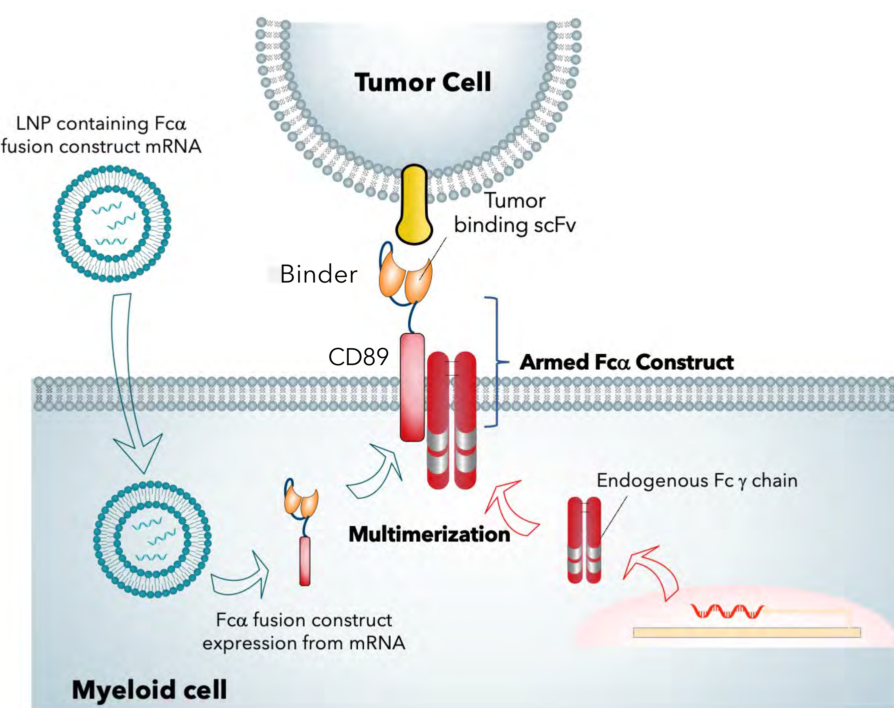
Hongyun Zhao, Michael Gorgievski, Neha Diwangi, Edward Cochran, Shannon Argueta, Yuxiao Wang, Bruce McCreedy, Thomas Prod'homme and Daniel Getts

Myeloid Therapeutics, 300 Technology Square Suite 203, Cambridge, MA 02139

Abstract

Immunotherapy has revolutionized cancer treatment. However, for the majority of patients with advanced solid tumors, sustained clinical benefit has yet to be achieved. Myeloid cells such as monocytes and macrophages readily accumulate in tumors, in some cases contributing up to 75% of the tumor mass. Reprogramming circulating and tumor associated myeloid cells to activate their ability to elicit anti-tumor adaptive immunity by phagocytosis, cytokine secretion and antigen presentation is an attractive approach to harness and orchestrate systemic anti-tumor immunity. It remains challenging to specifically target and activate myeloid cells in vivo. To overcome this hurdle, we have developed a novel in vivo myeloid cell engineering platform: Fcα receptor (FcαR) fusion proteins. Unlike other chimeric antigen receptors (CARs), the construct is engineered by fusing a tumor recognition scFv with the alpha chain of human Fc receptors (CD89). The stable expression and function of these receptors requires endogenously expressed Fc receptor gamma chain (FcRγ), a protein with limited expression to immune cells, mostly myeloid cells. Intravenous infusion of lipid-nanoparticle (LNP) encapsulating mRNA encoding the FcαR fusion protein results in the uptake of the LNPs and expression of the chimeric receptor fusion protein in myeloid cells. In immunodeficient xenograft models of hepatocellular carcinoma and triple negative breast cancer, delivery of LNP mRNA encoding GPC3 or TROP2 targeted FcαR fusion proteins resulted in anti-tumor efficacy, confirming the ability of this approach to program myeloid cells. Furthermore, in the B16/10 syngeneic melanoma model, treatment with the melanoma antigen gp75 targeted FcαR fusion protein was also associated with the initiation of broad systemic immune responses, characterized by infiltration of the TME by activated CD8$^+$ T cells, reduced tumor associated Tregs and activation of antigen presenting cells in spleen. When infused in cynomolgus monkeys, anti-TROP2 LNP led to cell surface expression of anti-TROP2 CAR and were not associated with significant modulation of safety readouts. Together these studies highlight the potential of FcαR fusion proteins delivered directly in vivo to program myeloid cells to recognize and kill cancer.

Introduction



Fcα fusion construct forms a multi-chain complex with the endogenous FcRγ chain, which is required for its stable cell surface expression.

This fully assembled armed Fcα fusion construct is expressed at the cell surface where it can recognize tumor cell surface target and activate myeloid cells' anti-tumor activity

Methods

- Syngeneic B16/F10-OVA model: 0.2 x 10^6 B16/F10-OVA cells were implanted s.c. in C57Bl/6 Females (6-8 wk-old).
- HCC1954 triple negative breast cancer (TBNC) xenograft model: 2 x 10^6 human (h)TROP2$^+$ HCC1954 cells were implanted s.c. in NSG Females (6-8 wk-old).
- All injections (Vehicle and LNP) were performed intravenously (i.v.). All treatments were initiated when tumor volume was >50 mm^3.
- P values: * p ≤ 0.05; ** p ≤ 0.01, *** p ≤ 0.001, ****p ≤ 0.0001

Results

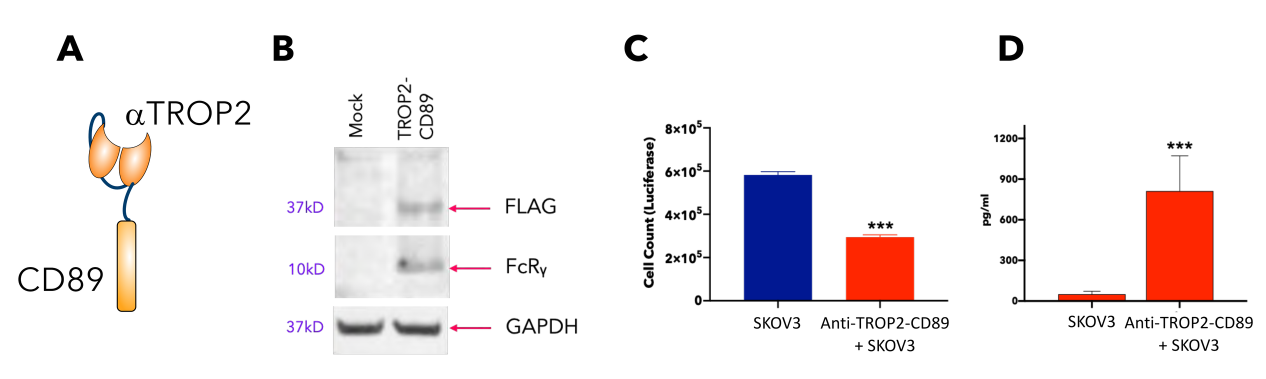


Figure 1: CAR-CD89 constructs pair with FcRγ leading to tumor killing and cytokine secretion. (A) Schematic of anti-TROP2-CD89 CAR. (B) Association of anti-TROP2-CD89 with FcRγ was assessed by co-immunoprecipitation. THP-1 cells (myelomonocytic) were transfected with FLAG-tagged anti-TROP2 CD89 mRNA. Anti-TROP2 CD89 was pulled down using anti-FLAG. Western Blot against FcRγ demonstrated the association. (C) PBMC transfected with the anti-TROP2-CD89 construct were co-cultured with the TROP2$^+$ ovarian carcinoma SKOV3-Luc cell line. Cell killing was addressed by measuring luciferase intensity. Cytokine secretion was addressed by Luminex. Statistical analysis: unpaired t-test.

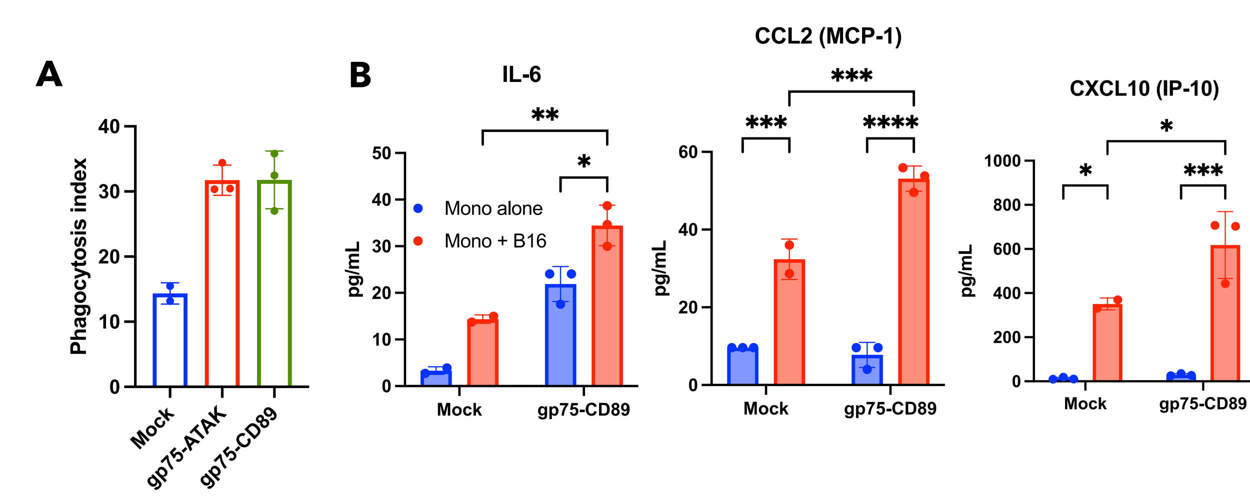


Figure 2: Antigen specific activity in vitro of CAR-CD89. (A) Ly-6C$^+$ monocytes purified from bone marrow were electroporated with either the gp75-ATAK mRNA, or the gp75-CD89 mRNA. Mock-transfected monocytes were used as negative controls. Antigen-specific phagocytosis was measured by incubating transfected monocytes with gp75$^+$ B16/F10 cells. Phagocytic index was calculated as: % phagocytic monocytes/% total monocytes x 100. (B) Antigen-specific cytokine/chemokine secretion was assessed by Luminex in supernatants from a 24h co-culture between transfected monocytes and B16/F10 cells. Mock-transfected monocytes co-cultured with B16/F10, mock-transfected monocytes and transfected monocytes alone were used as controls. Statistical analysis: 2-way ANOVA.

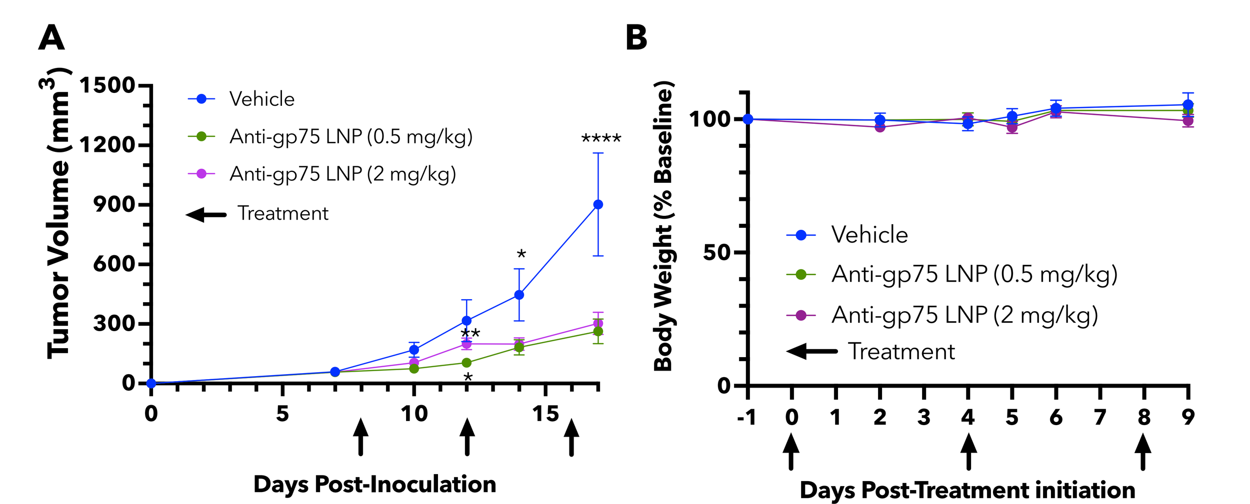


Figure 3: Potent anti-tumor activity in the syngeneic B16/F10 melanoma model. (A) Mice (n=5/group) were injected i.v. Q4D with either anti-gp75 LNP or PBS (Vehicle). (B) Body weight. Values are calculated over baseline (randomization). Statistical analysis: 2-way ANOVA.

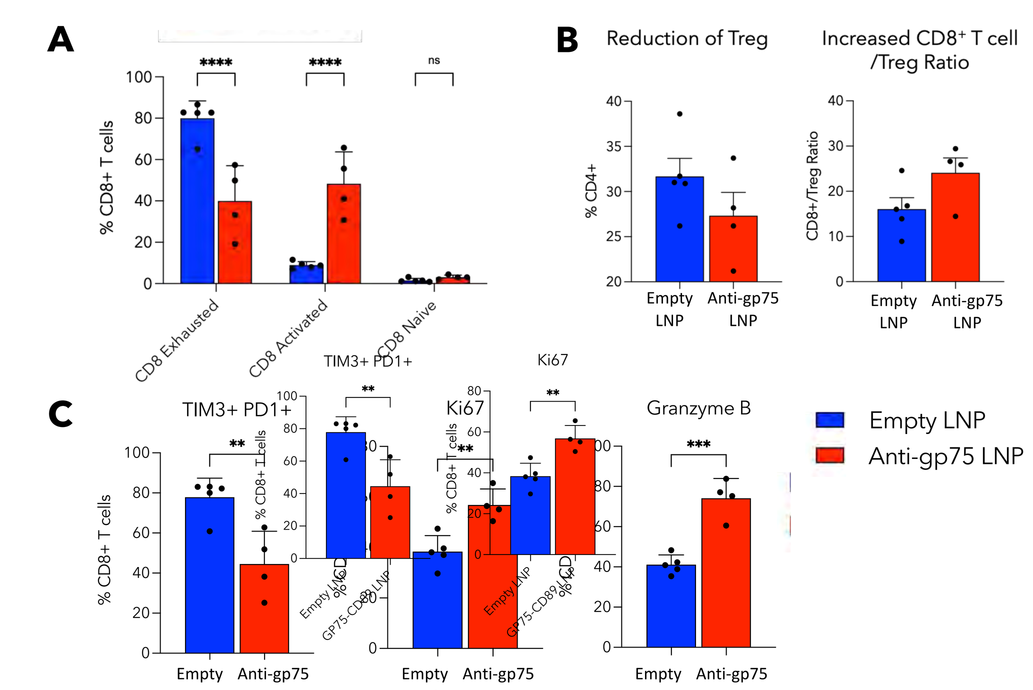


Figure 4: Anti-gp75-CD89 LNP increased activation and cytolytic activity of CD8$^+$ T cells in tumor. (A) Treatment with anti-gp75 LNP (2 mg/kg) significantly reduced the percentage of PD1^{hi} TOX$^+$ exhausted CD8$^+$ T cells and, conversely, significantly increased the frequency of activated CD8$^+$ T cells. Empty LNP were used as controls. (B) Significant reduction of the frequency of CD4$^+$ FoxP3$^+$ CD25$^+$ regulatory T cells (Treg) and increased CD8$^+$/Treg ratio. (C) Significant increase of the frequency of proliferating CD8$^+$ T cells (CD8$^+$ Ki67$^+$) and cytolytic CD8+ T cells (Granzyme B$^+$). Statistical analysis: one-way ANOVA.

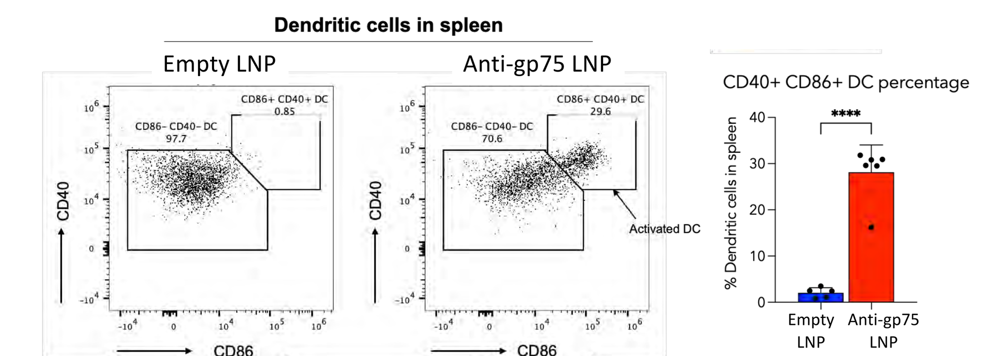


Figure 5: Treatment with anti-gp75 LNP increased activation markers for dendritic cells in the spleen. Splenocytes were isolated and analyzed by flow cytometry. DC were CD45$^+$ CD11c$^+$ MHCII$^+$. CD40 and CD86 expression indicates activation of dendritic cells in spleen. Statistical analysis: unpaired t-test.

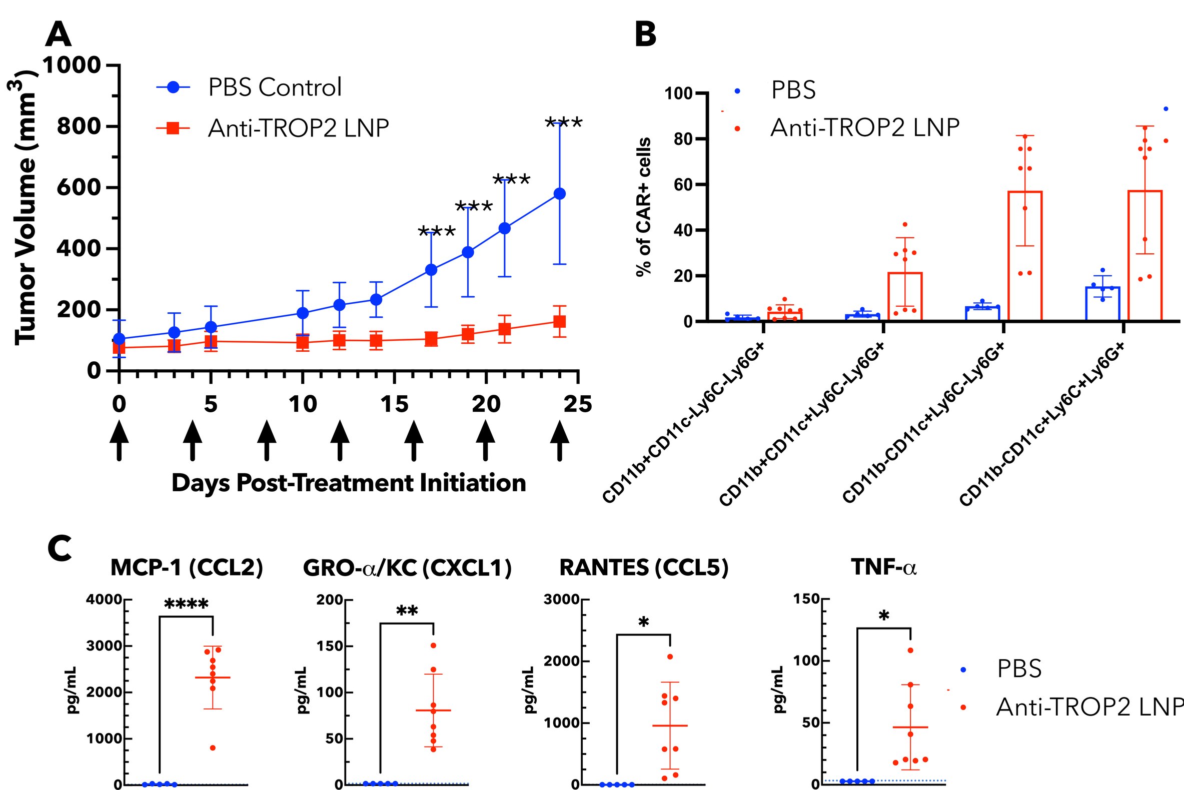
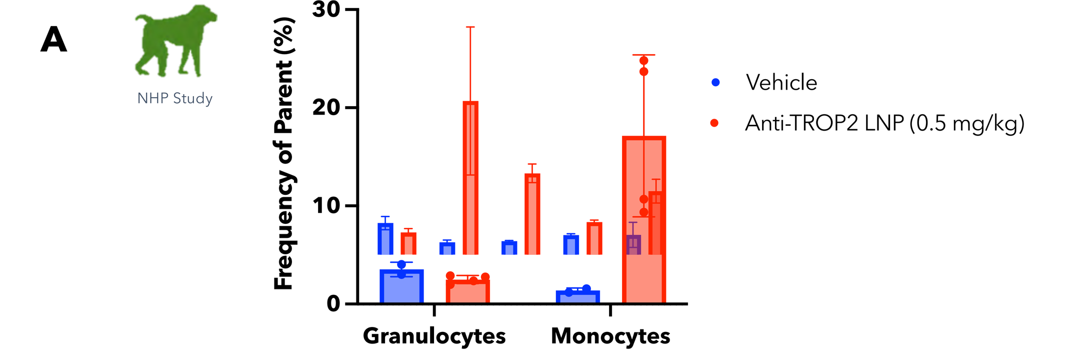


Figure 6: Fcα fusion construct delivered via LNP shows anti-tumor efficacy and myeloid cell specific CAR expression in a TNBC xenograft model. (A) Mice were injected i.v. Q4D or Q7D with either anti-hTROP2 CAR LNP (2 mg/kg; n=9), or PBS (n=5). (B) Expression of the anti-TROP2 CAR was assessed by flow cytometry in splenocytes from (A). (C) Luminex analysis of serum harvested 24h after the last injection. Statistical analysis: unpaired t-test.



B

		Predose		24h		168h	
		Vehicle	MT-302 (0.5 mg/kg)	Vehicle	MT-302 (0.5 mg/kg)	Vehicle	MT-302 (0.5 mg/kg)
Hematology	WBC	7.78	9.6725	12.8	5.4425	7.22	11.545
	RBC	6.12	6.0225	5.76	5.4175	5.725	5.9725
	PLT	347	350.5	8.7	302	290.5	426.75
	RETIC	18.6	33.675	8.45	28.1	55.7	82.1
	Neut	3.4	3.7675	3.18	3.07	3.69	5.1975
	Lymph	3.23	5.165	0.55	1.86	2.585	5.4675
	Mono	0.48	0.5	0.56	0.3975	0.62	0.595
	Eos	0.63	0.17	0.02	0.045	0.235	0.1975
	Baso	0.01	0.0225	0	0.025	0.045	0.0275
Serun chemistry	ALB	4.4	4.025	4.5	4	4.2	4.15
	TP	7.1	7.525	7.4	7.375	7	7.65
	ALP	91	343.75	116	353.75	101	317.25
	ALT	28	54.5	62	65	40.5	38.75
	AST	38	48	108	77	34.5	43.75
	TBIL	0.2	0.2	0.4	0.25	0.2	0.175
	CHOL	178	124	181	120.5	170.5	128.5
	TRIG	92	36.75	43	33.75	64.5	37
	GLOB	2.7	3.525	2.9	3.375	2.8	3.5
	A/G	1.6	1.15	1.6	1.175	1.5	1.2
	B/C	15	28.25	18	26.25	25	25.25
Cytokines/Chemokines	IFNγ	3.4	6.6	3.8	4.7	4.8	4.4
	TNFα	78.4	923.8	71.4	556.6	81.8	458.8
	IL-8	5924.3	2587.9	1145.6	3323.9	1801.1	3435.2
	CCL4 (MIP-1β)	9.2	13.9	10.2	17.6	12.8	15.8
	IL-12	19.4	47.7	12.7	28.5	37.1	46.5
	CCL2 (MCP-1)	584.1	440.9	1380.1	1467.2	601.3	380.2
	IL-1RA	27.8	39.5	48.6	835.0	44.0	38.7
	IL-1β	1.1	2.0	1.0	1.4	1.2	1.1
	IL-6	19.0	2.0	11.5	7.4	20.3	2.1
	IL-2	5.5	10.8	5.1	6.6	8.5	6.6

Figure 7: Infusion of anti-TROP2 LNP in cynomolgus monkey showed potent cell specific expression & was not associated with any increase in safety markers. Non-naïve cynomolgus monkeys were infused with anti-TROP2 LNP (0.5 mg/kg; n=2 males and n=2 females) or PBS (n=2 males and n=1 female) for 1h. (A) Anti-TROP2 CAR expression was addressed from lysed whole blood at 12h in myeloid cells (CD66abce$^+$ neutrophils and CD11b$^+$ monocytes). (B) Hematology (whole blood), serum chemistry and cytokine secretion (serum) were studied at predose, 24h and 168h post infusion.

Conclusions

- Stable expression of the scFv-CD89 fusion requires endogenous Fc receptor γ-chain (**Figure 1**), which is only present in immune cells, particularly myeloid cells.
- Expression of the gp75-CD89 construct in murine monocytes allows antigen-specific phagocytosis and secretion of soluble factors (**Figure 2**).
- Intravenous delivery of mRNA encoding Fcα fusion construct targeting the gp75 melanoma antigen shows potent anti-tumor activity in the B16/F10 melanoma syngeneic model (**Figure 3**).
- Mechanistically, delivery of Fcα fusion construct resulted in changes in tumor microenvironment, including a reduction of the frequency of Treg, reduction of exhausted CD8$^+$ T cells, and activation of cytolytic function of CD8$^+$ T cells (**Figure 4**). Fcα fusion construct delivery also increased the expression of activation markers in dendritic cells in spleen (**Figure 5**).
- In immunodeficient xenograft model of TROP2$^+$ breast cancer, delivery of LNP mRNA encoding TROP2 targeted Fcα Receptor Fusion constructs showed significant anti-tumor activity, whether when administered i.v. every 4 days or weekly (**Figure 6**).
- Infusion of anti-TROP2 LNP in cynomolgus monkeys resulted in cell surface expression of the CAR, mainly in monocytes. Administration of 0.5 mg/kg anti-TROP2 LNP was safe did not lead to modulation of safety readouts (**Figure 7**).