Intratumoral injection of SD-101, a novel interferogenic TLR9 agonist, unlocks the full potential of PD-1 blockade

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Background

- Therapies blocking the PD-1-PD-L1 pathway have produced durable tumor responses in a wide variety of human cancer types. However, such responses occur only in a minority of patients receiving PD-1 or PDL-1 blockade monotherapy.
- Recent studies have found that the response to anti-PD-1 mAb, Pembrolizumab, is highly correlated with the presence of infiltrating T cells in the tumor. (*Nature*, 2014; 515 (7528): 568).
- The current challenge for increasing the response rate to PD-1/PDL-1 blocking therapies is to understand how to transform a non-permissive tumor microenvironment into a fully competent one, capable of producing optimal antitumor response.
- One promising strategy is intratumoral injection of a TLR9 agonist, inducing Type I IFN and subsequent production of ligands for the T cell homing receptor CXCR3.
- SD-101 belongs to the CpG-C class of CpG-ODN and was selected to stimulate very high levels of Type I IFNs as well as inducing maturation of plasmacytoid dendritic cells to antigen-presenting cells. Results from studies in non-human primates and phase I/II studies, demonstrated that subcutaneous administration of SD101 generates a response characterized by a dose dependent induction of Type I IFN regulated genes in PBMC.

SD-101: A CpG-C Class Oligonucleotide Designed for Cancer

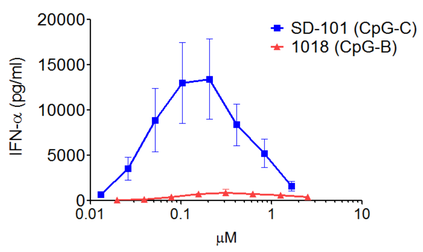
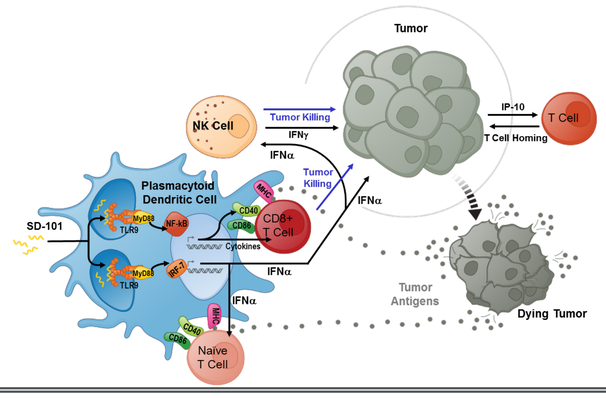


Figure 1: SD-101 induces high level of IFN-α in human PBMC PBMC were isolated from healthy donors and stimulated for 24hr with increasing amount of SD-101 (CpG-C class) and 1018 (CpG-B class).

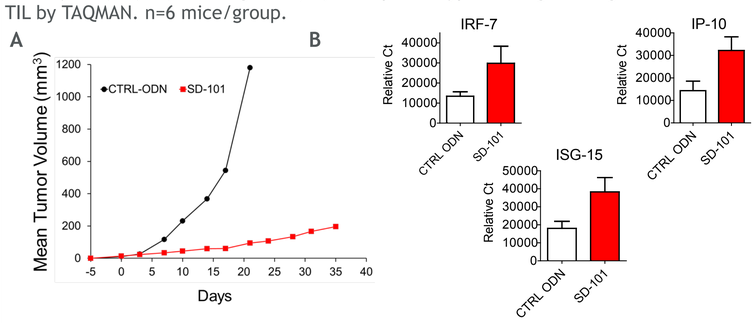
SD-101 cellular mechanisms of action

Both innate and adaptive immune response are increased by intratumoral injection of SD101. SD101 induces high levels of Type I IFN secretion from Plasmacytoid Dendritic cells (PDC) which is a potent immunomodulatory cytokine, able to boost NK cytotoxic activity and to induce recruitment of T cells. In addition, SD101 induces PDC maturation and ability to cross-present tumor associated antigens, promoting CD8 T cells response.



Intratumoral injection of SD-101 Induces a Sustained Type I IFN Gene Signature in the Tumor Environment

Figure2: Tumor model: CT26 colon carcinoma, injected at Day -5. From Day 0 (average tumor size 4mm) SD-101 or non-CpG CTRL-ODN were administered intratumorally (50μg/injection) twice a week for three weeks. On Day 25 (3 days after last treatment) the group injected with the CTRL-ODN was euthanized due to excessive enlarged tumors. On day 35 (13 days after last treatment) the group injected with SD-101 was euthanized. Tumors were collected and processed to extract tumor infiltrating leukocytes (TIL). A) tumor growth; B) example of Type IFN regulated genes measured in TIL by TAQMAN. n=6 mice/group.



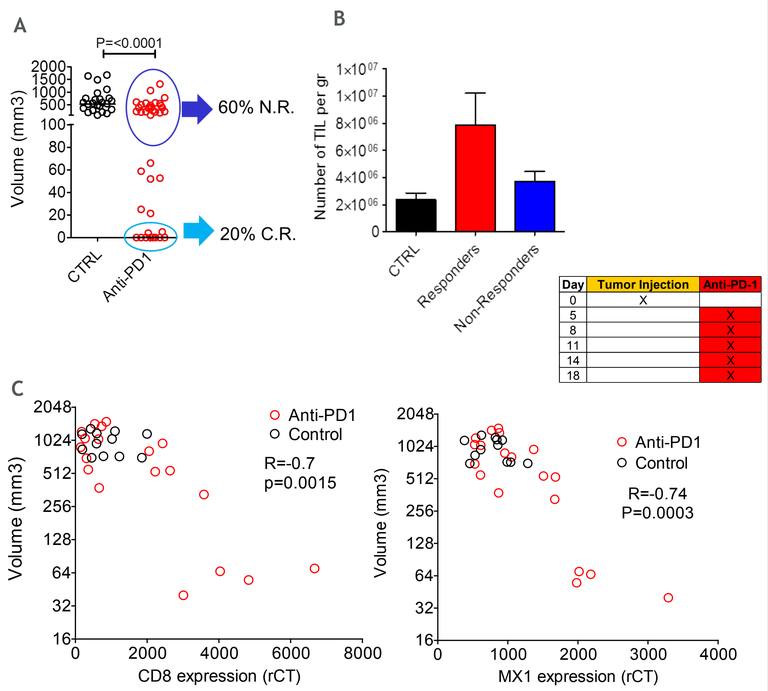
OBJECTIVE

To test the ability of intratumoral SD-101 to reverse tumor escape from anti-PD1 treatment.

Results

Experimental model:

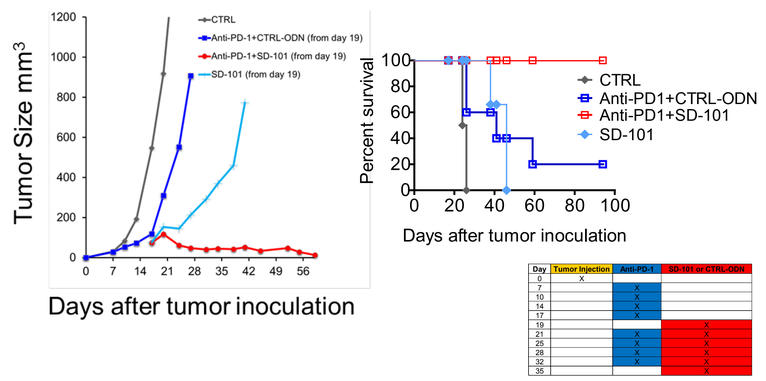
Figure 3: Anti-PD1 therapy in mouse CT-26 tumor model mimics human outcomes
A) Mice transplanted with the CT26 colon carcinoma produce a heterogeneous response to systemic PD-1 blockade, with 60% of tumors progressing at the same rate as in untreated mice and only 20% of the tumors being completely rejected.
B) Mice that respond to anti-PD-1 treatment have increased number of leukocytes infiltrating the tumor (TIL) and the response correlates with CD8 T cell infiltration and Type I IFN genes levels (panel C shows gene expression level in whole tumors of CD8 and MX1 gene, as an example of a Type I IFN regulated gene).
Treatment schedule as depicted in table. Anti-PD1 (200μg/injection i.p.).
Mice were euthanized 2-4 days after last anti-PD-1 treatment.



Day	Tumor Injection	Anti-PD-1
0	X	
5		X
8		X
11		X
14		X
18		X

Intratumoral SD-101 with continued anti-PD-1 treatment reverses tumor escape from anti-PD-1 therapy and leads to long-term, immune-mediated control of tumor growth

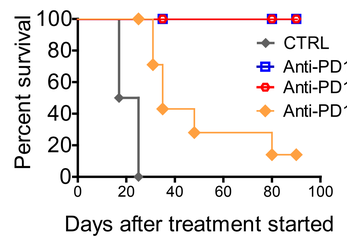
Figure 4: Tumor model: CT26 colon carcinoma, injected at Day 0. Anti-PD-1 treatment started when tumor reached 5 mm (day 7). After 4 anti-PD-1 (day 19), mice that already rejected tumor were taken out of the study and the remaining were randomized and started receiving IT injections of SD-101 or of a non-CpG control sequence (CTRL-ODN); in both groups anti-PD-1 treatment was continued as depicted in the table. A separate group of mice with the same tumor size, not pre-treated with anti-PD1, started receiving SD101 alone. This group was necessary to compare the effect of SD-101 when added to anti-PD-1 treated mice, versus SD-101 given in mice not-treated with anti-PD-1. Anti-PD1 was used at 200μg/injection. SD101 or CTRL-ODN was used at 50μg/injection IT as depicted in the table. Shown is a representative experiment of at least three, each performed with 5-10 mice per group.
Similar results were obtained using an anti-PDL-1 blocking Ab.
All mice that rejected in response to anti-PD-1 plus SD-101 combination rejected a second challenge of tumor cells given 80-100 days after first challenge.



Day	Tumor Injection	Anti-PD-1	SD-101 or CTRL-ODN
0	X		
7		X	
10		X	
14		X	
17		X	
19			X
21		X	X
25		X	X
28		X	X
32		X	X
35			X

CD8+ T cells but not CD4+ T cells are required for the efficacy of anti-PD-1 plus SD-101 combination treatment.

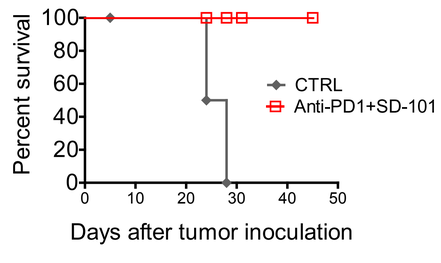
Figure 5: Experiment was performed as described in Figure 4. Experimental details are as as depicted in the table. n=7 mice/group.



Day	Tumor Injection	Anti-PD-1	SD-101	Anti-CD8	Anti-CD4
0	X				
5		X			
9		X			
12		X			
14				X	X
15		X	X	X	X
16				X	X
19		X	X	X	X
22		X	X	X	X
26		X	X	X	X
29		X	X	X	X

Combination therapy of Anti-PD-1 plus SD-101 induces systemic immune response able to reject contralateral tumors

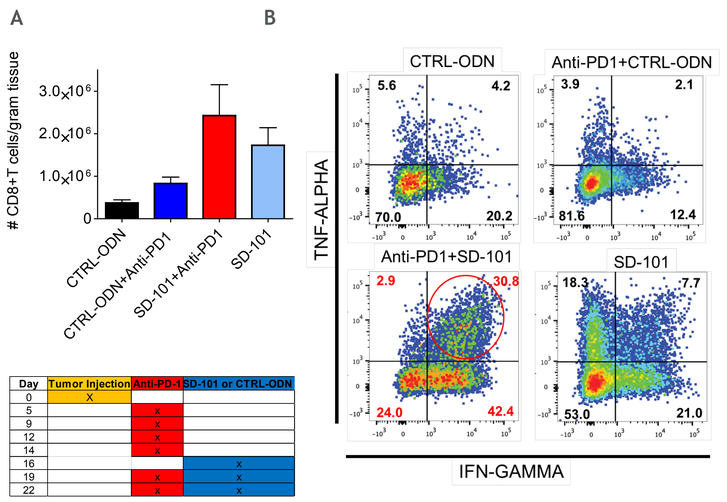
Figure 6: Survival of mice bearing 2 s.c. tumors (right and left flank) that received systemic anti-PD-1 and IT SD-101 only in the left tumors. Experimental details as depicted in the table. n=9 mice/group



Day	Tumor Injection	Anti-PD-1	SD-101
0	X		
5		X	
7		X	
11		X	
14		X	X
19			X
21			X
23		X	X
26		X	X

SD-101 strongly induces infiltration and activation of polyfunctional tumor infiltrating CD8+ T cells

Figure 7: Experiment was performed as described in Figure 4. Experimental details are as depicted in the table. Tumors were harvest 4 days after last injection (day 26) and processed for enrichment of TIL analyzed by flow cytometry. A) Shows the number of total CD8 T cells per gram of tumor. B) TIL were re-stimulated for 3 hr with PMA and Ionomycin in the presence of BFA and then analyzed by intracellular staining and flow cytometry (gated on CD8 T cells). An increased number of CD8 T cells co-expressing TNF-α and IFN-γ infiltrate tumors treated with the combination therapy. One representative experiment out of three is shown.



Day	Tumor Injection	Anti-PD-1	SD-101 or CTRL-ODN
0	X		
5		X	
9		X	
12		X	
14		X	
16			X
19		X	X
22		X	X

Conclusions

Intratumoral injection of SD-101 induces significant Type I IFN production and CD8 T cell infiltration in the tumor microenvironment.

SD-101 is able to reverse tumor escape from anti-PD-1 treatment leading to a CD8 T cell mediated tumor rejection.

These data provide a strong rationale for the clinical assessment of SD-101 in combination with agents blocking the PD-1/PD-L1 pathway.