SO-C101 displays strong anti-tumor effect in TC-1 and TRAMP-C2 tumor mice and in combination with PD-1 blockade prevents tumor development in a NK and CD8⁺ T cells dependent manner

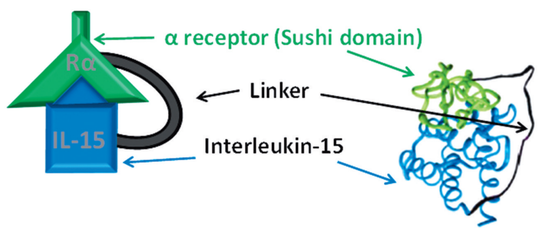
Irena Adkins[1,2], Romana Mikyskova[3], Nada Podzimkova[1,2], Lenka Palova Jelinkova[1,2], Guy de Martynoff[4], David Bechard[4], Ulrich Moebius[1], Milan Reinis[3], Radek Spisek[1,2]

[1]SOTIO, Prague, Czech Republic; [2]Department of Immunology, 2nd Faculty of Medicine and University Hospital Motol, Charles University, Prague, Czech Republic; [3]Laboratory of Immunological and Tumour models, Institute of Molecular Genetics of the ASCR, v.v.i., Prague, Czech Republic; [4]Cytune Pharma, Nantes, France

Introduction

SO-C101 (RLI-15) is a superagonist fusion protein of interleukin (IL)-15 and the IL-15 receptor α (IL-15Rα) sushi⁺domain. SO-C101 is designed to bypass the need for endogenous IL-15Rα, thereby leveraging the activity of IL-15 *in vivo* on target immune cells and reducing the toxicity of IL-15 as such. SO-C101 was previously shown to exhibit a potent anti-metastatic activity in Renca, B16F10 melanoma and delayed tumor growth in T cell-based mouse tumor models (CT26, MC38). Here we investigated the anti-tumor efficacy in predominantly natural killer (NK)-cell based mouse tumor models TC-1 and TRAMP-C2. We showed that SO-C101 monotherapy was effective in the treatment of established TC-1 tumors, which was dependent on the presence of both NK and CD8⁺ T cells, but not CD4⁺ T cells. In an early treatment setting, SO-C101 significantly decreased the rate of tumor development also in dependence on NK and CD8⁺ T cells. SO-C101 effectively reduced tumor growth in TRAMP-C2 mice in early and advanced treatment settings. However, only in combination with anti-PD-1 antibody treatment was tumor development prevented in the majority of mice. This effect was durable, and new tumor development was further significantly delayed after a tumor cell re-challenge, which suggests the involvement of memory T cells despite an important NK cell role in anti-tumor efficacy in these models. The efficacy of SO-C101 and anti-PD-1 treatment was not dependent on CD4⁺ T cells, but mainly on NK and CD8⁺ T cells. Interestingly, SO-C101 and anti-PD-1 treatment in double NK/CD8⁺ T cell-depleted mice decreased tumor growth which suggests an involvement of other immune cell populations in the anti-tumor efficacy. SO-C101 stimulated the proliferation and the cytotoxic activity of NK cells and memory CD8⁺ T cells without significant expansion of regulatory T cells. These data show the importance of various immune cell populations during SO-C101 monotherapy and the treatment in combination with anti-PD-1 antibodies, and set a base for further complex analysis of SO-C101 behavior. The therapeutic potential of SO-C101 is currently being tested in an ongoing Phase I clinical study in cancer patients.

SO-C101 (RLI-15)



- SO-C101 (211 amino acids) is a fusion protein that consists of the N-terminal-sushi⁺ domain of the human IL-15Rα (77 amino acids) covalently coupled via a non-immunogenic linker of Glycine-Serine residues (20 amino acids) to the mature IL-15 sequence (114 amino acids) (Mortier et al., 2006).
- SO-C101 acts as a selective and potent agonist of the IL-15 pathway through IL-15Rβγ thereby inducing proliferation and activation of memory CD8⁺ T cells, NK cells, γ/δ T cells and NKT cells *in vitro* and *in vivo* and exerts significantly increased anti-tumor efficacy in various mouse cancer models over IL-15 (Bessardet al., 2009; Desboiset al., 2016)

Figure 1

SO-C101 (RLI-15) decreases the tumor development rate and growth in TC-1 model which depends on the presence of both NK and CD8⁺ T cells

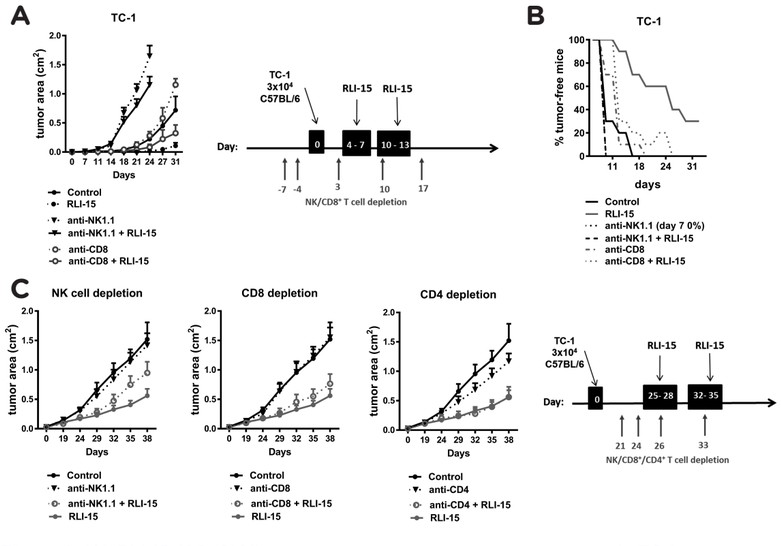


Figure 1. **(A)** RLI-15 (SO-C101) decreases the tumor development rate in TC-1 mouse tumor model in dependence on NK and CD8⁺ T cells. Mice were treated *s.c.* with RLI-15 at 2 mg/kg for 4 consecutive days over 2 weeks according to the scheme. Depletion antibodies for NK/CD8⁺/CD4⁺ T cells were used **(B)** The percentage of tumor-free mice over time shows that treatment with RLI-15 decreases the rate of the tumor development. Depletion of NK cells markedly accelerates the TC-1 tumor growth (no tumor-free mice at day 7). **(C)** RLI-15 decreases the tumor growth of established TC-1 tumors in dependence on NK and CD8⁺ T cells, but not CD4⁺ T cells. Mice were treated *s.c.* with RLI-15 (SO-C101) at 2 mg/kg for 4 consecutive days over 2 weeks according to the scheme. Data represent n=2-3.

Figure 2

SO-C101 (RLI-15) expands immune cells in tumor, lymph nodes and spleen and activates NK and CD8⁺ T cytotoxicity genes in TC-1 tumor mouse model

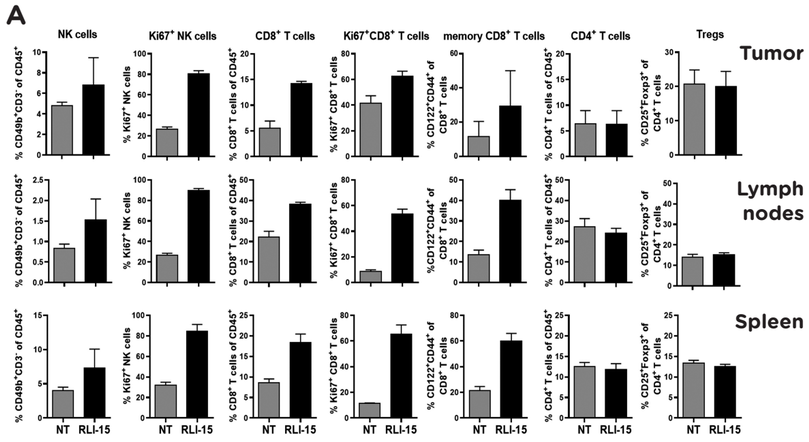


Figure 2. (A) RLI-15 (SO-C101) monotherapy at 2 mg/kg, D1-D4 induces proliferation (Ki67⁺) and expansion of immune cells populations such as NK cells, CD8⁺ T cells, specifically memory CD8⁺ T cells, but not CD4⁺ T cells and T regulatory cells (Tregs) in the tumor, inguinal lymph nodes and the spleen at Day 5. **(B)** Nanostring (nCounter Mouse PanCancerImmune Profiling Panel) analyses showed that RLI-15 monotherapy activates NK and CD8⁺ T cell cytotoxicity genes in tumor, inguinal lymph nodes and spleen. RLI-15 – mice with established tumors were treated with RLI-15 (*s.c.*, 2 mg/kg, D1-D4), samples collected at Day 5 after the first dosing (n=2-3); control – TC-1 tumor-bearing mice without treatment (n=3).

Figure 3

SO-C101 (RLI-15) decreases tumor growth of established TRAMP-C2 tumors and expands CD8⁺ T cells and NK cells, but not Tregs

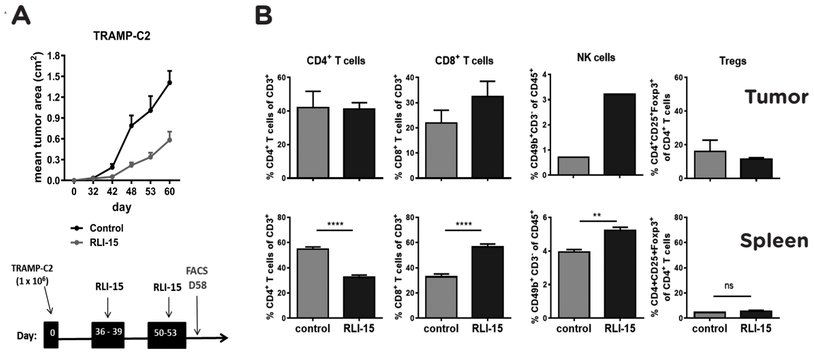


Figure 3. (A) RLI-15 (SO-C101) decreases tumor growth of established TRAMP-C2 tumors. Mice were treated *i.p.* with RLI-15 at 1 mg/kg for 4 consecutive days over 2 weeks according to the scheme. **(B)** Immune cell populations were analyzed 5 days after the last RLI-15 dose by flow cytometry from the tumors and spleens. The increase in NK and CD8⁺ T cells but not CD4⁺ T cell and Tregs was shown in the tumor and in the spleen of TRAMP-C2 tumor-bearing mice. Data represent values of 2 independent experiments from 5 mice. Some tumors were pooled.

Figure 4

SO-C101 (RLI-15) and anti-PD-1 treatment prevents tumor development in the majority of TRAMP-C2 mouse and delays tumor growth after re-challenge

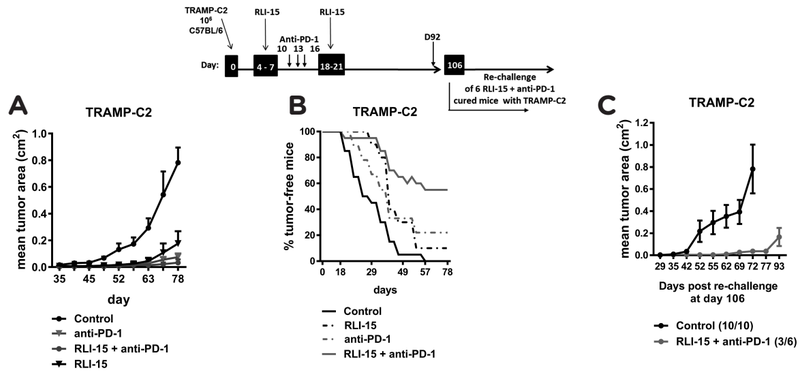


Figure 4. (A) RLI-15 (SO-C101), anti-PD-1 and their combination decreases tumor volume in early treatment TRAMP-C2 mouse model. Mice were treated *i.p.* with RLI-15 at 1 mg/kg and with anti-PD-1 at 12.5 mg/kg according to the scheme. **(B)** RLI-15 + anti-PD-1 prevents tumor development in the majority of TRAMP-C2 mice in contrast to monotherapies. **(C)** RLI-15 + anti-PD-1 treatment significantly delays tumor development after re-challenge with TRAMP-C2 tumor cells. Data represent summary of n=2-3.

Figure 5

SO-C101 (RLI-15) and anti-PD-1 mediated inhibition of TRAMP-C2 tumor development is dependent mainly on NK and CD8⁺ T cells

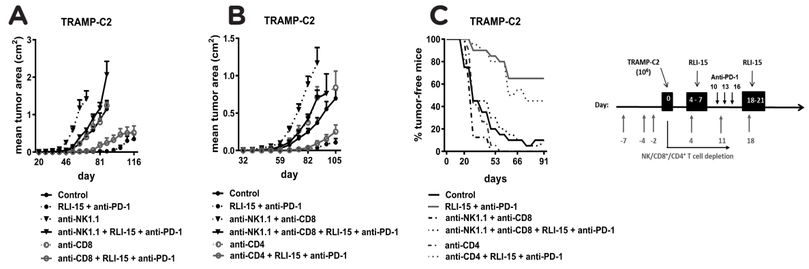


Figure 5. (A,B) RLI-15 (SO-C101) + anti-PD-1 treatment decreases the tumor growth in early treatment TRAMP-C2 mouse model which is dependent mainly on NK and CD8⁺ T cells, but not CD4⁺ T cells. Mice were treated *s.c.* with RLI-15 at 1 mg/kg and with anti-PD-1 at 12.5 mg/kg according to the scheme. Antibodies for depleting NK/CD8⁺/CD4⁺ T cells were used. **(C)** RLI-15 + anti-PD-1 decreases the tumor development rate in TRAMP-C2 mice in dependence mainly on NK and CD8⁺T cells as shown by the percentage of tumor-free mice.

Conclusions

- **SO-C101 decreases the rate of tumor development and growth in the TC-1 mouse tumor model which depends on the presence of both NK and CD8⁺ T cells.**
- **SO-C101 monotherapy induces proliferation and expands immune cells in tumor, lymph nodes and spleen, and activates NK and CD8⁺ T cytotoxicity genes in the TC-1 tumor model**
- **SO-C101 decreases tumor growth of established TRAMP-C2 tumors and expands CD8⁺ T cells and NK cells, but not Tregs.**
- **SO-C101 and anti-PD-1 treatment prevents tumor development in the majority of TRAMP-C2 mouse and delays tumor growth after the re-challenge.**
- **SO-C101 and anti-PD-1 mediated inhibition of TRAMP-C2 tumor development is dependent mainly on NK and CD8⁺ T cells.**

References

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For more information please contact Irena Adkins, adkins@sotio.com