Expanded Therapeutic Window of Potent Bispecific PD-L1 T Cell Engager with Novel Best-in-Class CD3 Binder

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Abstract: 8249

Introduction

Bispecific T cell engagers (TCE) are potent, off the shelf immune modulators that have been hugely successful against hematologic malignancies and are beginning to demonstrate utility in solid tumors. PD-L1 targeting bispecific TCE with an SP34 variant CD3 binder showed exceptionally potent activity against intracranial tumor models in mice. To further expand the potential therapeutic index, we engineered SCRI-6, a novel, high affinity, non-polyreactive CD3 binder, into a next generation PD-L1 TCE and compared the SP34 and SCRI-6 versions for efficacy and tolerability.

Methods

In vitro activity of the TCEs were assessed in T cell killing assays targeting iRFP labeled NCI-H1975 tumor cells at an effector to target ratio of 5:1. The pharmacodynamics of systemically administered PD-L1:CD3 TCE was interrogated using SP34 or SCRI-6 CD3 binders against NCI-H1975-GFP/ffLuc tumor cells intracranially implanted in adult NOD.Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/SzJ (NSG) mice from Jackson Laboratories, modeling metastatic lung cancer. Activity of SP34 or SCRI-6 TCEs with a murine PD-L1 binder was evaluated in mice transgenic for human CD3ε obtained from GenOway.

Results

PD-L1:CD3^{SCRI-6} TCE molecule induced T cell killing of the NCI-H1975 lung cancer cells in dose dependent manner with maximal tumor death following 72-hr exposure (Fig 1A). SCRI-6 TCE was equally active *in vitro* to the SP34 version with EC_{50} values of 5.02 and 3.55pM, respectively (Fig 1B). Next, we examined the efficacy of PD-L1:CD3^{SCRI-6} TCE in a metastatic xenograft model intracranially implanted NCI-H1975-GFP/ffLuc tumor cells. 100% of the mice remained healthy (Fig 2A) and the TCE eliminated bioluminescent tumor signal (Fig 2B) at doses from 0.03 to 2.12 mg/kg (the highest dose tested). Doses of 0.0005-0.008 mg/kg did not alter tumor growth, and the median survival was similar to the negative control group, establishing the lower limit of efficacy for this TCE. A dose titration from 0.01 to 5 mg/kg of both the SP34 and SCRI-6 TCE in human CD3ε transgenic mice showed pharmacodynamic responses with similar, modest and transient loss of body weight (Fig 3A and 3B) and transient depletion of circulating lymphocytes at doses of 0.4-5mg/kg (Fig 3C and 3D). Cytokines associated with cytokine release syndrome (IL-6, IFNγ and TNFα) were found to be elevated to higher levels in SP34 TCE treated mice compared to those in SCRI-6 TCE mice (Fig 3E, 3F and 3G). INFγ was significantly higher in the SP34 TCE at 5, 2.5, and 0.1mg/kg (p=0.03, 0.05, and 0.01 respectively), TNFα at 5mg/kg (p=0.003), and IL-6 at 1.3mg/kg (p=0.03). Suggesting a higher tolerated threshold for SCRI-6 based TCE. In the NCI-H1975 brain metastases model, while treatment with either the SCRI-6 or SP34 TCEs resulted in the survival of 100% of the mice (Fig 4A), treatment with SCRI-6 TCE induced complete and durable remissions for the entirety of the study as seen in the bioluminescent signal, whereas remissions tended to be variable and transient with the SP34 version (Fig 4B).

Potent T Cell Killing Activity of TCE with Novel CD3 binder, SCRI-6

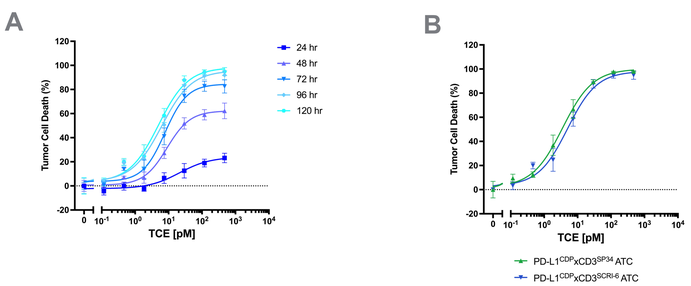

Figure 1: PD-L1:CD3^{SCRI-6} TCE has potent anti-tumor activity against NCI-H1975. **(A)** Time course of TCE-mediated T cell killing (E:T ratio of 5:1). **(B)** TCE with CD3^{SCRI-6} binder has comparable activity to tool molecule with CD3^{SP34} in *in vitro* T cell killing assay with EC_{50} values of 5.02 and 3.55pM, respectively.

TCE with SCRI-6 Improved Survival in Xenograft Model of Metastatic Lung Cancer

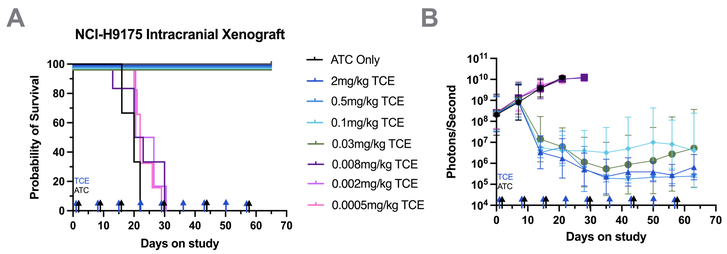

Figure 2: PD-L1:CD3^{SCRI-6} eliminated intracranial tumors in dose-dependent manner. **(A)** Kaplan-Meyer curve for NSG mice bearing intracranially implanted NCI-H1975 xenografts treated with $7.5x10^6$ ATCs alone or a dose titration of PD-L1:CD3^{SP34}+ATC. **(B)** Bioluminescence of implanted tumors in response to TCE treatment.

Reduced Cytokines in Response to SCRI-6 Compared to SP34 in Humanized CD3 Mice

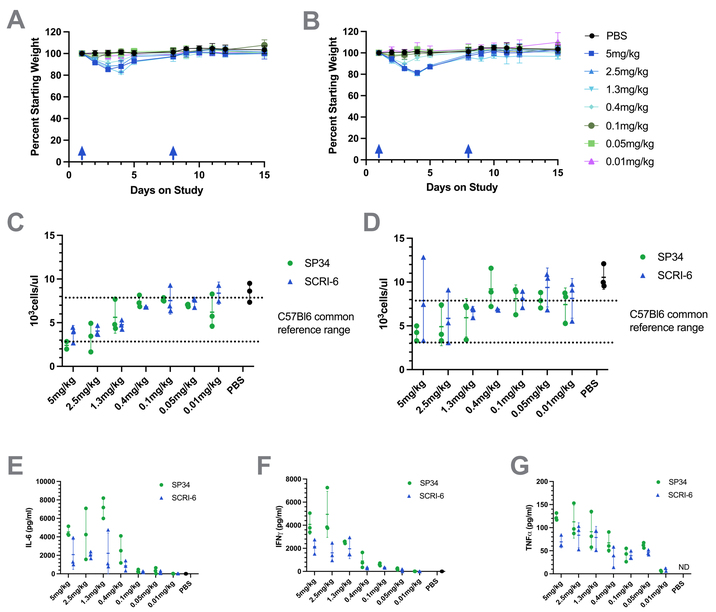

Figure 3: TCE administration is well tolerated in transgenic humanized CD3 mice. **(A-B)** Modest weight loss was observed in GenOway hCD3ε mice following administration of PD-L1:CD3^{SCRI-6} TCE (A), similar to that observed with CD3^{SP34} (B). **(C-D)** Circulating lymphocytes at day 7 (C) and day 15 (D) after TCE administration. **(E-G)** Serum concentration of IL-6 (E), INFγ (F) or TNFα (G) 4 hours after TCE administration comparing SCRI-6 to SP34 CD3 binders.

SCRI-6 TCE Induced Complete Remission in Xenograft Model of Metastatic Cancer

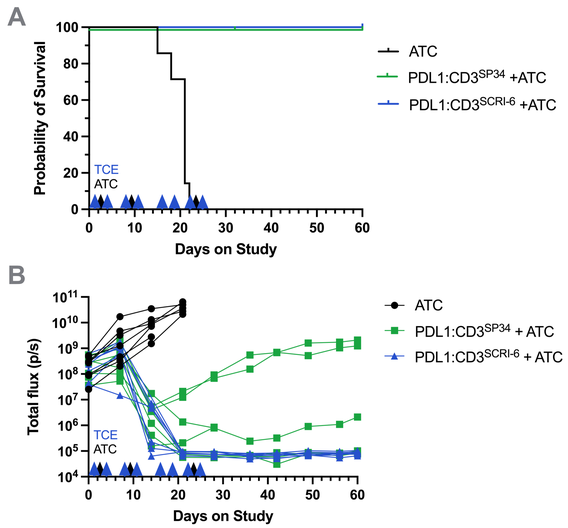

Figure 4: Efficacy of SCRI-6 vs SP34 TCEs against intracranial NCI-H1975 xenografts. **(A)** Kaplan-Meyer curve for mice bearing NCI-H1975-GFP/ffLuc xenografts treated with ATCs alone, PD-L1:CD3^{SP34}+ATC, or PD-L1:CD3^{SCRI-6} TCE+ATC. **(B)** Bioluminescence of implanted tumors in response to TCE treatment.

Conclusions and Future Directions

- PDL1:CD3^{SCRI-6} TCE is highly efficacious with a minimum effective dose of 0.03mg/kg against an intracranial xenograft model of metastatic lung cancer.
- PD-L1 targeted TCE was well tolerated in humanized CD3 mice.
- SCRI-6 TCE showed reduced induction of cytokines associated with CRS compared to SP34
- Systemic treatment with SCRI-6 TCE induced complete and durable tumor remission in contrast to incomplete and transient remissions in mice treated with the same dose of SP34 TCE.
- Together these data have shown an expanded therapeutic window between efficacy and safety of the PD-L1:CD3^{SCRI-6} TCE compared to PD-L1:CD3^{SP34}, supporting progression to clinical development.

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