FS120, an OX40/CD137 tetravalent bispecific dual agonist antibody, synergistically increases the antitumor activity of anti-PD-1 in preclinical studies

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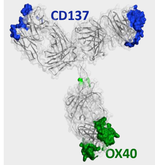
Background

Immune checkpoint inhibitors have demonstrated durable clinical responses and an increase in overall survival for some patients with cancer. Next generation cancer immunotherapies, such as tumor necrosis factor receptor superfamily (TNFRSF) agonists, have potential to further improve on this success.

FS120 is a tetravalent bispecific antibody targeting OX40 and CD137 (4-1BB), currently being evaluated in a Phase I clinical trial (NCT04648202). FS120 activates CD4⁺ and CD8⁺ T cells by concurrent binding to both targets via an FcγR-independent mechanism[1]. In preclinical tumor models, FS120 induced T cell proliferation and cytokine production associated with significant tumor regression, better than that observed with a monoclonal antibody combination. Here, we demonstrate the ability of FS120 to improve anti-PD-1 mAb induced T cell activity, increasing tumor growth inhibition and survival, in syngeneic mouse tumor models, compared to monotherapy.

1. FS120 and PD-1 mAb Mechanism of Action



1. *Activation of effector cells (CD8⁺ T cells and NK cells)*
2. *Stimulation of helper activity (CD4⁺ T cells)*
3. *Potentially decreases Treg activity, making them unstable and fragile*

Key Properties:

- CD137 agonist with K_D = 0.2 nM
- OX40 agonist with K_D = 0.2 nM
- Dual conditional agonist activity dependent on coexpression of OX40 and CD137 in the tumor
- Natural IgG format for ease of manufacture and Fc gamma binding null for safety

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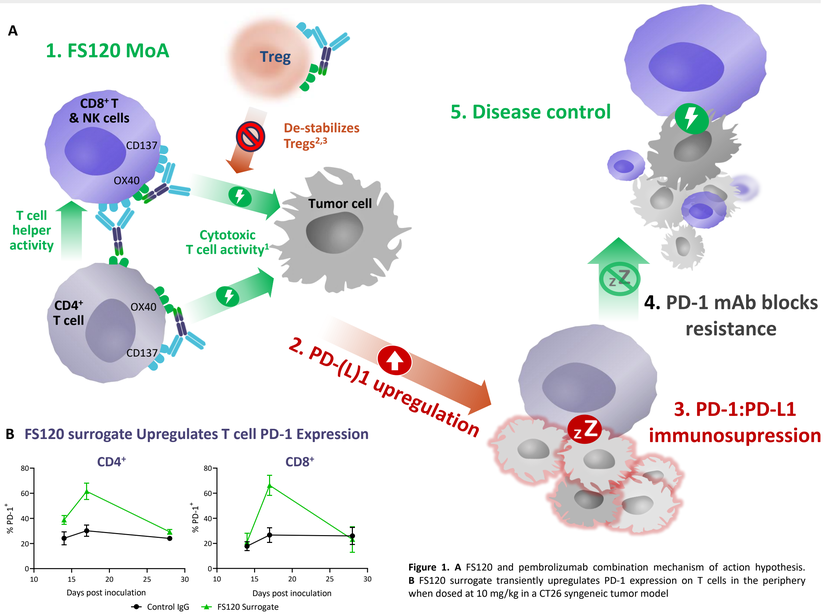

Figure 1. A FS120 and pembrolizumab combination mechanism of action hypothesis. **B** FS120 surrogate transiently upregulates PD-1 expression on T cells in the periphery when dosed at 10 mg/kg in a CT26 syngeneic tumor model

2. FS120 Enhances Pembrolizumab Activity in Human Primary *in vitro* Assays

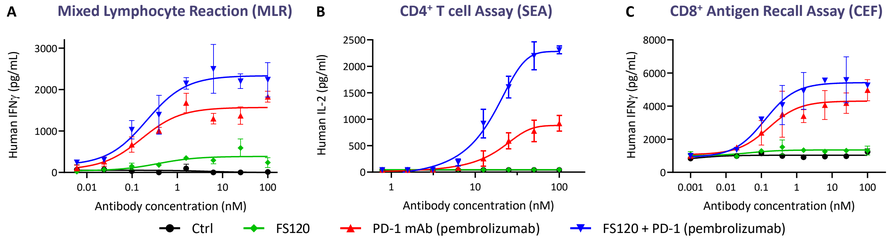

Figure 2. Combination activity of FS120 and anti-PD-1 (pembrolizumab) in human primary immune cell functional assays. A Human IFNγ release in a human MLR consisting of allogenic iDC and expanded CD4⁺ T cell co-cultured for 5 days, in response to isotype control, FS120, pembrolizumab or the combination. **B** Human IL-2 release on 4 days co-cultured expanded CD4⁺ T cell + iDC Staphylococcus aureus enterotoxin A (SEA) assay in response to isotype control, FS120, pembrolizumab or the combination. **C** Human IFNγ release in HLA class II-restricted CEF peptide pool antigen recall assay in response to isotype control, FS120, pembrolizumab or the combination.

3. Combination Significantly Improves Survival Benefit Versus Either Monotherapy in a PD-1 mAb Refractory Tumor Model

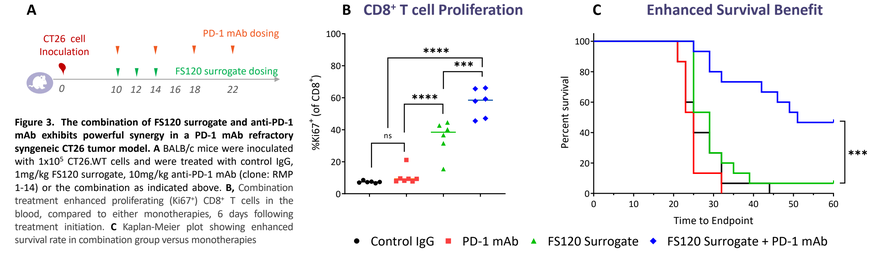

Figure 3. The combination of FS120 surrogate and anti-PD-1 mAb exhibits powerful synergy in a PD-1 mAb refractory syngeneic CT26 tumor model. A BALB/c mice were inoculated with 1x10⁵ CT26.WT cells and were treated with control IgG, 1mg/kg FS120 surrogate, 10mg/kg anti-PD-1 mAb (clone: RMP 1-14) or the combination as indicated above. **B,** Combination treatment enhanced proliferating (Ki67⁺) CD8⁺ T cells in the blood, compared to either monotherapies, 6 days following treatment initiation. **C** Kaplan-Meier plot showing enhanced survival rate in combination group versus monotherapies

4. PD-1 mAb Lowers the Threshold for FS120 Surrogate Anti-tumor Activity

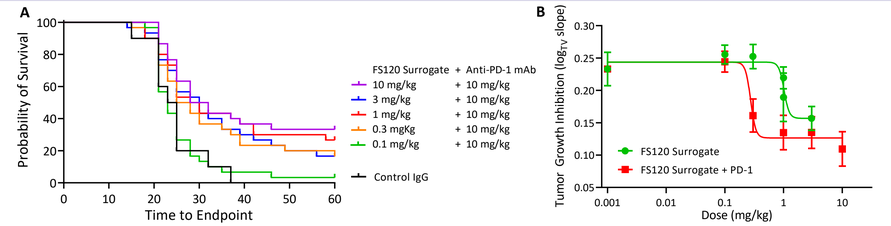

Figure 4. The synergistic activity of combining FS120 surrogate and PD-1 mAb at different dose levels in mice. BALB/c mice were dosed with 10mg/kg PD-1 mAb and FS120 surrogate in the range of 0.1mg/kg to 10mg/kg 10 days after CT26 cell inoculation. The dosing plan was as descripted above in figure 3. **A** Kaplan-Meier plot for survival indicating a threshold change when adding PD-1 mAb in combination with FS120 surrogate. **B** Combination of FS120 surrogate and PD-1 mAb inhibits tumor growth to a greater extent compared to FS120 surrogate monotherapy, shifting the IC50

5. FS120 Surrogate Enhances Anti-PD-1 mAb Mediated Effector Cell Cytotoxicity

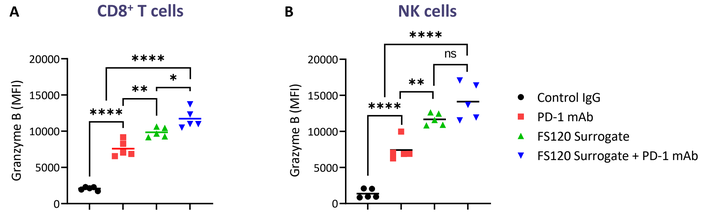

Figure 5. Granzyme B expression by CD8⁺ T cells and NK cells from the periphery of CT26 tumor-bearing mice. CT26 tumor-bearing mice were dosed with isotype control, FS120 surrogate, PD-1 mAb or the combination. Blood PBMCs were collected on day 17 after inoculation. Cells were treated with protein transport inhibitor brefeldin A for 4 hours followed by flow cytometry staining and analysis. **A** Granzyme B expression by CD8⁺ T cells. **B** Granzyme B expression by NK cells.

6. Combination Induces a Strong, yet Transient, Proinflammatory Response

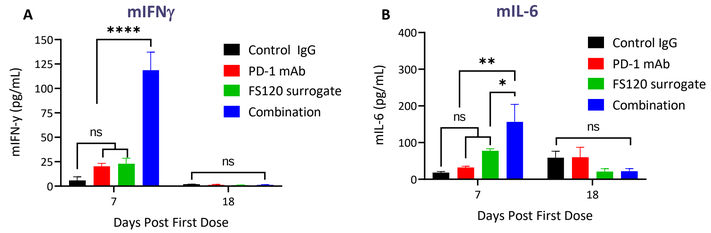

Figure 6. The combination of FS120 surrogate and PD-1 mAb significantly, yet transiently, increased IFNγ and IL-6 in serum from CT26 tumor-bearing mice. CT26 tumor-bearing mice were treated with isotype control antibody, FS120 surrogate, PD-1 mAb or the combination as described in figure 3. Serum was analysed via meso scale discovery (MSD) for **A** mouse IFNγ and **B** mouse IL-6. Representative graphs from assays performed in duplicate; data are presented as mean ± SEM.

Conclusion

FS120 combination with anti-PD-1 enhances T cell activity in multiple human primary immune assays. In combination with an anti-PD-1, FS120 surrogate increased the antitumor efficacy with pharmacodynamic changes related specifically to T cell activation, when compared to monotherapies.

These data support the development of FS120 in combination with anti-PD-1 mAb in patients with cancer resistant to checkpoint therapy.

FS120 is currently being evaluated in a Phase 1 monotherapy dose escalation trial (NCT04648202) which aims to identify a safe, tolerated and pharmacologically active dose of FS120 for exploration in future clinical studies as monotherapy, and in combination with other agents.

Following determination of a safe and pharmacologically active dose, FS120 will be evaluated in combination with KEYTRUDA®, with the potential for early demonstration of clinical activity in specific tumor subtypes[4]. F-star expects to provide a progress update on the FS120 monotherapy accelerated dose titration cohorts later this year and plans to initiate the KEYTRUDA combination cohorts in the second half of 2022, following completion of the FS120 monotherapy dose escalation.

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[2]T reg fragility: A prerequisite for effective antitumor immunity? Curr Opin Immunol. 2016 doi: 10.1016/j.coi.2015.12.009
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