An Innovative Human Platform for Targeted Delivery of Bispecific Interleukins to Tumors

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BACKGROUND

Introduction: Combination approaches using stimulatory cytokines, checkpoint inhibitors, chemotherapy, and/or radiation therapy are known to improve overall survival in cancer patients. Recombinant interleukins (IL) have had mixed clinical success due to short pharmacokinetics (pK), inefficient tumor targeting and more frequent dosing, leading to toxicities. To address these issues, we have developed a novel platform that delivers immunomodulators in either a mono- or bispecific format. The platform consists of a fully human albumin-binding scFv domain (F_HAB) with an improved pharmacokinetic profile that enhances tumor targeting by binding over-expressed FcRn, GP60, and SPARC, as well as a dose-sparing effect that decreases toxicity risk and broadens the therapeutic index.

Interleukins-12, -15, and -18 are among the most potent inducers of anti-tumor activity and have been evaluated in numerous clinical studies. Sonnet's bispecific drug candidates are constructed with IL-12 on the F_HAB platform and include IL12-F_HAB-IL15 and IL18-F_HAB-IL12. These bispecific molecules span a broad range of mechanisms of action that bridge innate and adaptive tumor immunity. The "cold" immunosuppressive B16-F10 melanoma tumor model was used to compare the efficacy of the bispecific candidates administered in a single intravenous dose. These bispecific molecules are produced in CHO cells using an intensified perfusion manufacturing process that allows for rapid scale-up for commercial manufacturing.

Methods: Female C57BL/6 mice received a subcutaneous inoculation of 0.2 × 10^6 B16F10 cells. Grouping and treatments were initiated when the mean tumor volume reached approximately 90 to 100 mm^3. Animals from all groups were dosed once via an intravenous injection into the tail vein.

MECHANISM OF ACTION

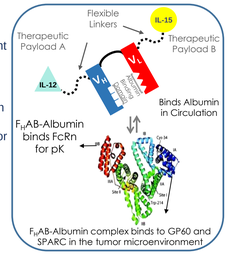
Asset: SON-1010 (IL12-F_HAB)
State: Preclinical/Phase 1, 1Q 2022

Disease Area: Solid Tumors – NCSCL, Head & Neck, Melanoma, Breast

Target / MOA: Asset delivery and targeting by albumin binding mechanism via the F_HAB domain results in the accumulation of SON-1010 in the tumor microenvironment (TME) of select solid tumors, resulting in improved penetration and retention, and thus, increased efficacy.

Product Description: SON-1010 has enhanced pK via binding to FcRn and improved tumor delivery. The design potentiates accumulation in and retention by the TME, primarily through albumin binding to over-expressed tumor proteins, GP60 and SPARC.

- Fully human sequence – reduced immunogenicity
- Produced in CHO – glycosylated
- Small size and linear flexibility enhance tumor penetration
- Simple plug-and-play platform for rapid asset development



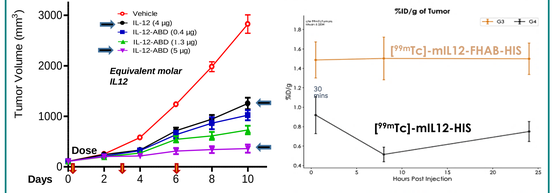


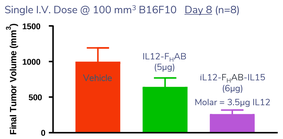
Figure 1. B16F10 tumor-bearing mice, doses 3x of IL-12 (4µg) and IL12- F_HAB (5µg) molar equivalent amounts demonstrate IL12- F_HAB had superior bioactivity in reducing tumor progression. Labeled mIL12-F_HAB-HIS molecule accumulated ~2-3x fold higher than the mIL12-HIS (without F_HAB domain) within the tumor.

Bispecific IL12-F_HAB-IL15

Sonnet's Bispecific Construct – SON-1210

Synergistic Biologic Activity:

- IL-12: ↑ IL-15 alpha receptor, ↑ IFN, ↑ NK/T cells, ↑ TH1 and ↓ T reg
- IL-15: ↑ IL-12 beta 1 receptor, ↑ NK cells, ↓ CD8 memory loss by apoptosis



Bispecific IL18-F_HAB-IL12

Sonnet's Bispecific Construct – SON-1410

Synergistic Biologic Activity:

- IL-18: ↑ IL-12 receptor, ↑ IFNγ, ↑ TH1, NK, CD8 cells *infiltrating into tumors – FACS data*
- IL-12: ↑ IL-18 receptor, ↑ IFNγ,
- IL-12 with IL-18 ↑ CXCL9 & CXCL10 by 50-fold

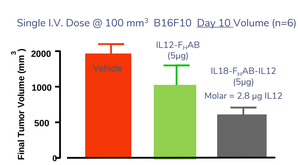
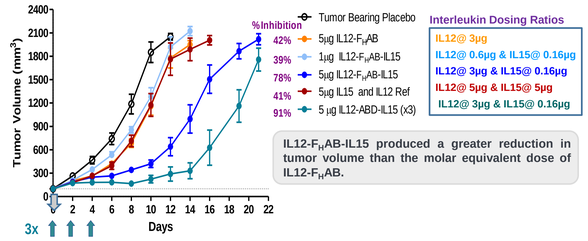
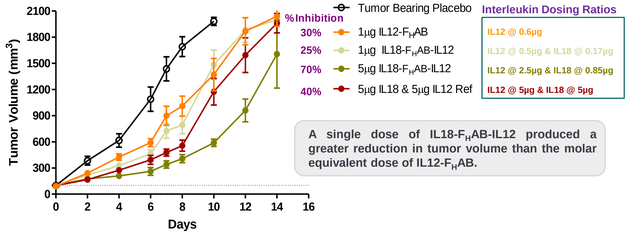


Figure 2. Monospecific vs Bispecific interleukin constructs linked with F_HAB based on their biology show better reduction in tumor growth when administered in a bispecific format.



F_HAB-IL15 produced a greater reduction in tumor volume than the molar equivalent dose of IL12-F_HAB.

Figure 3. B16F10 tumor-bearing mice treated with Monospecific IL12-F_HAB vs Bispecific IL12-F_HAB-IL15 or IL18-F_HAB-IL12 produced a greater reduction in tumor volume at molar equivalent dose of IL-12 with a single dose. One set at 5µg was dosed 3x.



A single dose of IL18-F_HAB-IL12 produced a greater reduction in tumor volume than the molar equivalent dose of IL12-F_HAB.

Figure 4. IL18-F_HAB-IL12 produced a greater reduction in tumor volume compared to a 3.5x lower molar dose of IL12-F_HAB. Dosed at 5µg, IL18-F_HAB-IL12 having a ↓2x and ↓6x lower levels of IL-12 and IL-18, respectively, and compared to reference (no F_HAB), IL18-F_HAB-IL12 showed 70% vs 40% tumor reduction, thus displaying synergistic biological activity of the bispecific.

RESULTS

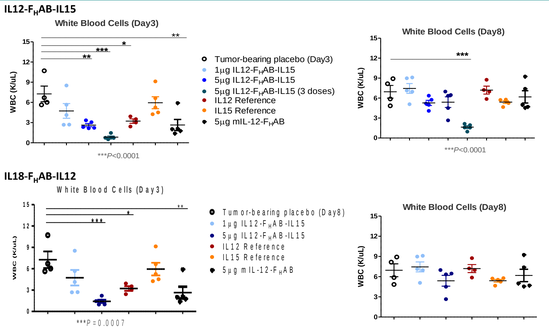


Figure 5. Hematology profile of both bispecific constructs after a single dose on Day 3 showed similar decreases in total white blood cell counts, with each profile rebounding to placebo levels at Day 8.

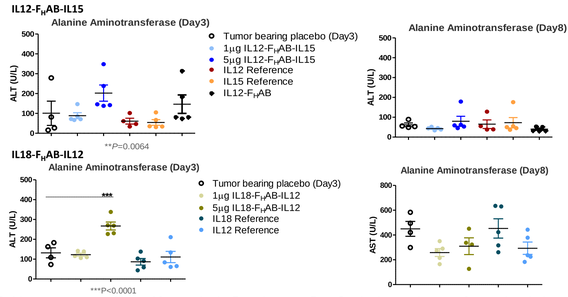


Figure 6. Liver enzyme profiles. After a single 5µg dose on Day 3, alanine aminotransferase levels increased but decreased to placebo levels by Day 8. Observed aspartate aminotransferase levels did not change.

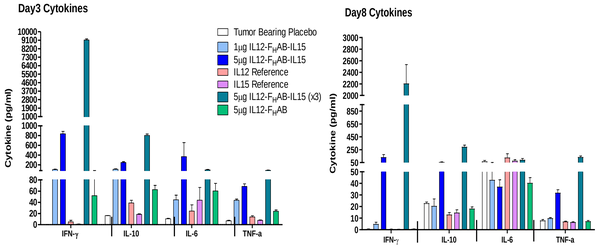


Figure 7. Cytokine profiles of the IL12-F_HAB-IL15 construct after a single Day 3 dose showed a strong increase in IFN-γ levels compared to the IL-12 reference with a concomitant decrease by Day 8. IL-6 levels were the same for all constructs compared to tumor placebo while IL-1ß, IL-2, IL-4 and IL-5 showed no change compared to placebo.

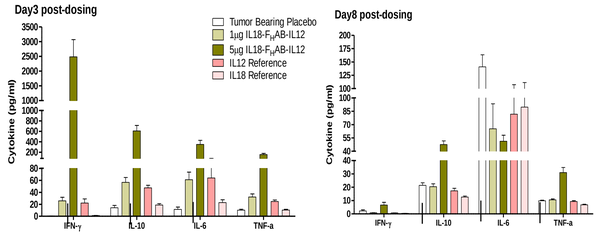


Figure 8. Cytokine profile of the IL18-F_HAB-IL12 construct after a single Day 3 dose showed a strong increase in IFNγ levels with decreased levels by Day 8. At Day 8, IL-6 levels were the same for all constructs compared to placebo while IL-1, IL-2, IL-4 and IL-5 showed no change compared to placebo.

FACS Analysis

Comparison of Efficacy Tumor & Spleen Immune Cell Type Day 5, TV ~400mm³	IL12-F_HAB (1µg) Inhibition 37%		IL12-F_HAB-IL15 (5µg) Inhibition 78%		IL18-F_HAB-IL12 (5µg) Inhibition 65%	
	Tumor	Spleen	Tumor	Spleen	Tumor	Spleen
Cell Population						
T cells	0.8	1.0	0.5	0.9	1.2	0.9
CD4+ T Cells	0.8	0.6	1.2	0.5	1.2	0.7
Th1 Cells	1.6	1.0	1.7	0.8	3.4	1.8
CD8+ T Cells	1.2	0.8	1.4	0.7	6.5	0.9
Cytotoxic CD8+, IFNγ	1.8	1.5	3.6	1.7	1.8	1.5
NK Cells	1.5	1.1	3.3	1.3	2.5	1.3
NK Cells, IFNγ	1.7	0.6	6.0	0.7	12.0	2.7
M1 Macrophages	1.4	2.9	1.4	3.0	1.8	3.2
M2 Macrophages	0.2	1.2	0.3	4.0	0.1	3.5
Regulatory (T Reg) Cells	0.9	1.2	0.6	0.8	1.7	1.6

Figure 9. Flow cytometry analysis of interleukin constructs. At Day 5 post single dose, an increase in immune stimulating cells was observed within tumors corresponding to a decrease in tumor volume. Also, there was a transition of M2 to M1 in the tumor. IL18-F_HAB-IL12 showed the strongest infiltration of immune cells into the tumor based on the biology of IL-18.

CONCLUSIONS

- All constructs showed statistically significant tumor size reduction compared to placebo or native interleukin at a 5µg dose: 67% for IL12-F_HAB-IL15 and 76% for IL18-F_HAB-IL12. At lower dose levels with similar efficacy, mice showed no body weight loss and exhibited reduced toxicity with transient adverse reactions that resolved by Day 8.
- Optimal synergistic efficacy occurred with the IL18-F_HAB-IL12 bispecific. Interestingly, IL-18 upregulates the IL-12 receptor (and vice versa) and increases levels of chemokines CXCL9 and CXCL10, which in turn, resulted in significant increases in activated NK, NKT, Th1 and cytotoxic CD8 T cells. Further, an increase in M1 and a converse decrease in M2 cells in tumor versus spleen was observed. These data support the potential for transitioning immunologically "cold" tumors to clinically responsive "hot" ones .
- These studies demonstrate that beyond the powerful anti-tumor effects of IL-12 evident in the monospecific IL12-F_HAB, in the bispecific format, IL-12 can synergize with other cytokines e.g., IL-18 and IL-15, to produce superior anti-tumor activity.
- Ongoing *in vitro* and *in vivo* studies will involve bispecific IL-F_HAB constructs used with various checkpoint inhibitors to further improve the immune stimulation and anti-tumor activities of novel combination therapies.