SITC Poster # P313
11/10/17

In vivo Effect of Albumin Binding Domains (ABD) Attached to Immune Modulators

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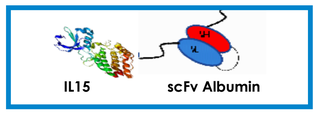
Introduction

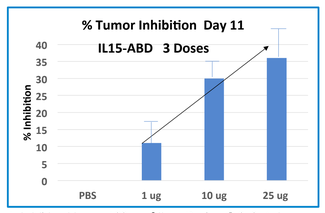
Small molecular weight (<50Kd) therapeutic proteins (eg., IFN, IL2, etc.) have had limited clinical success due to their short plasma half-lives, a result of first pass renal filtration, and to insufficient tumor targeting. Approaches to improve the serum half-life of these small MW therapeutic proteins include conjugation to polyethylene glycol (ie., PEG: eg., Peg-Intron, NKT-214) or formulations on the surface of or within nanoparticles. Albumin has also been successfully exploited as a carrier for small therapeutic proteins. Examples include the genetic fusion of Interferon to albumin (Albuferon) and the use of a multivalent albumin binding antibody fragment that has the capacity to bind serum albumin and also neutralize TNFa (Ozoralizumab). A potential advantage to the use of albumin as a drug carrier is that albumin is known to accumulate in inflamed and angiogenic tissues such as the tumor microenvironment, and may thus provide a means of passive targeting to the tumor.

We describe here the discovery of a fully human single chain variable antibody (scFv) construct (using a XOMA scFv human phage library) that has high binding affinity to serum albumin across species (mouse, human & *cynomologous* monkey), and retains the benefits of FcRn mediated recycling of albumin for extending serum half-life. This scFv Albumin Binding Domain (ABD) has been used to create genetic fusion protein constructs with several different small molecular weight therapeutic proteins (eg., recombinant interleukins such as IL12, and scFvs' against relevant immune-oncology targets, ie., TGFβ). In mouse models, these ABD constructs have extended serum half-lives, improved tumor accumulation and enhanced efficacy compared to their respective naked recombinant therapeutic protein. This poster will summarize the discovery of our scFv-ABD and preliminary studies with four different therapeutic scFv-ABD constructs that demonstrate the broad utility of this approach.

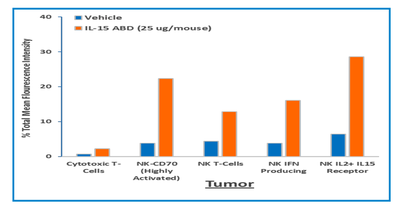
Figure 1

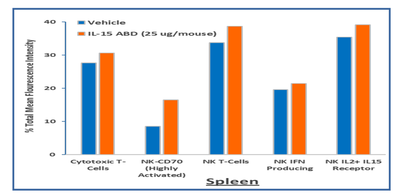


Effect of IL-15 ABD in B16F10 on Tumor Growth and on Lymphocyte Populations in Spleens and Tumors



Established tumor 100 mm^3 then Dosing @ 0, 2, 4 days



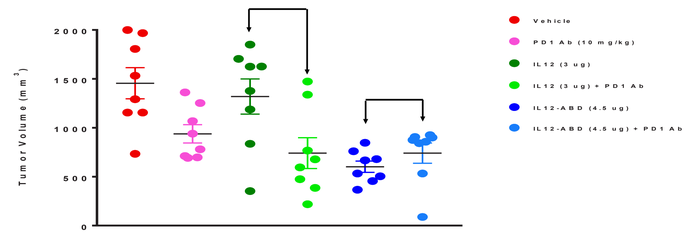


IL15-ABD showed reduction in TV and a 5-7 fold increase in the tumor NK cells (FACS data next slide)

Figure 5

IL12-ABD and IL12 in Combination with Anti PD1 Ab

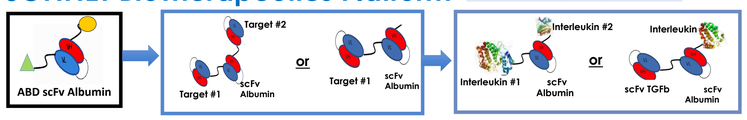
Single Dose (Tumors Established at 100 mm^3) – Day 8 Tumor Volume (n = 8)



IL12-ABD was more effective than treatment with either anti-PD1 or the molar equivalent dose of recombinant IL12. Moreover, IL12-ABD was as effective as the combination of recombinant IL12 plus anti-PD1 treatment. Interestingly, the addition of anti-PD1 Ab to recombinant IL12 improved the efficacy of either treatment alone, whereas, anti-PD1 treatment provided no further benefit to IL12-ABD

Figure 9

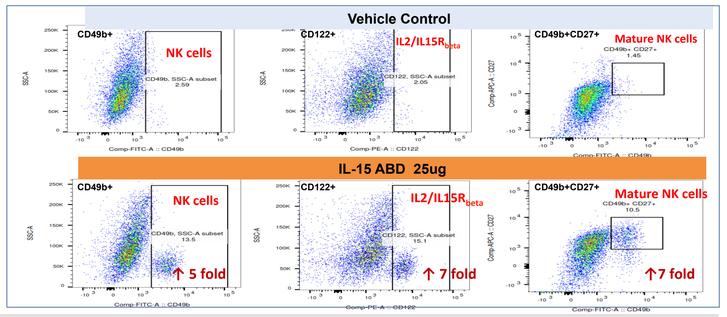
SONNET BioTherapeutics Platform (Bi or Tri targeting arms)



1. **Single-Chain Antibody (ScFv) Based**
   - **Flexibility** for multiple mechanisms of action
   - **Dual affinity targets** enhance specific tumor delivery
   - **Small size** enhanced tumor penetration
   - **Plug-and-play versatility** for target selection can *inhibit and/or stimulate*
2. **Albumin Binding Domain (ABD); scFv binds Human Serum Albumin (HSA)**
   - **Tumor delivery –** HSA is elevated in tumors & inflamed tissues
   - **Enhanced pharmacokinetics -** $T^{1/2}$ from minutes to days
3. **Recombinant Human (Hu) Proteins (Interleukins; scFv vs. TGFb)**
   - ABD – hu Interleukins (e.g. IL12 or IL15)
   - ABD – 2X hu Interleukins (e.g. IL15 –ABD- IL12)
   - ABD – scFv (e.g. TGFβ)

Figure 2

Effect of IL-15 ABD Treatment on Tumor Infiltration Lymphocyte Populations



Analysis of infiltrating lymphocyte populations by FACS show increases in NK cell populations within the tumor of IL15-ABD treated mice resulting in a reduction of tumor growth.

Figure 6

IL15-ABD-IL12 vs. IL12-ABD

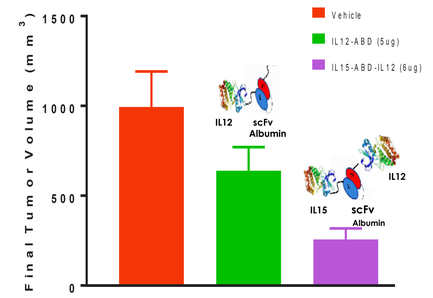
Single iv Dose (@ 100 mm^3 sc B16F10) Day 10 Tumor Volume (n=8)

Sonnet Tri-Specific Construct
IL15-ABD-IL12

Synergistic Biological Activity:

IL12 : ↑ IL15 alpha receptor, IFN, NK/Tcells, TH1 & ↓ Treg

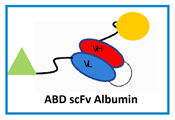
IL15 : ↑ IL12 beta 1 receptor, NK cells & ↓ CD8 memory loss



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

Figure 10

ABD Bio-Activity Selection Criteria to Ensure Increase in PK

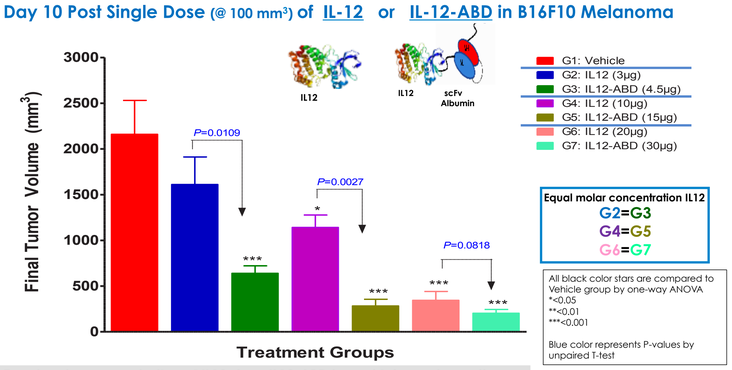


- ☑ **Binding to Human & Cyno Serum Albumin** *kD ~20 - 60 nM*
- ☑ **Binding to Mouse Serum Albumin** *kD ~10 - 30 nM*
- ☑ **Binding at low pH 5.5 & neutral pH7.2 for human, mouse albumin**
- ☑ **No competition with FcRn binding to serum albumin. In-vitro studies with ABD showed cell recycling with the FcRn hence should extend pK in-vivo (see Fig below)**
- ☑ **Binds Albumin in serum by immune-precipitation**
- ☑ **Human serum stability at 37C for 7-14 Days**
- ☑ **Immunogenicity tested by Antitope computer analysis**

XOMA (Library 47) full human scFv phage library was used to screen for a scFv by solid & solution panning against HSA, MSA and h/mTGFβ 1, 2 & 3.
ABD selection was based on meeting all of the above criteria.
Anti Albumin Binding Domain (ABD) A10M3 is a scFv: VH3/VL1, Tm ~60C, MW 26KD, pI 7.1
Anti TGFβ selection based on blocking TGF inducing of: Tregs proliferation, TGF→SMAD2 PO4, EMT & Migration
Anti TGF β (4D9M) is a scFv: VH3/VL1, Tm ~62C, MW 26KD, pI 5.3

Figure 3

Day 10 Post Single Dose (@ 100 mm^3) of IL-12 or IL-12-ABD in B16F10 Melanoma



Equal molar concentration of IL12 show IL12-ABD has a higher potency than IL12 in tumor reductions

Figure 7

Conclusions

1. A novel 26 Kd scFv antibody (ABD) that binds mouse, *cynomolgus* monkey and human serum albumin has been identified, and is being developed, as a platform for enhancing the pharmacokinetics and tumor targeting of small molecular weight therapeutic proteins (Figures 2-4).
2. Dose dependent increases in anti-tumor activity vs sc B16F10 mouse melanoma were demonstrated with either IL15-ABD or IL12-ABD constructs (Figures 5 & 7), producing a corresponding increase in tumor infiltrating NK cells following IL15-ABD treatment (Figures 6). FACs studies on cell infiltrates following IL12-ABD treatment are planned.
3. Molar equivalent doses of IL12-ABD were significantly more efficacious than naked recombinant IL12 vs. the B16F10 mouse melanoma model (Figure 7-9) and produced a corresponding increase in immune response as reflected by spleen weight and serum IFN, which was transient and had no effect on mouse body weight (Figure 8).
4. IL12-ABD was more effective than treatment with either anti-PD1 or the molar equivalent dose of recombinant IL12. Moreover, IL12-ABD was as effective as the combination of recombinant IL12 plus anti-PD1 treatment. Interestingly, the addition of anti-PD1 Ab to recombinant IL12 improved the efficacy of either treatment alone, whereas, anti-PD1 treatment provided no additional benefit to treatment with IL12-ABD, alone (Figure 9)
5. A first-in-class dual targeting tri-specific cytokine construct (IL15-ABD-IL12, 6ug/kg) was superior to the activity of a molar equivalent dose of IL12-ABD (5ug) vs. B16F10 *in vivo* (Figure 10).

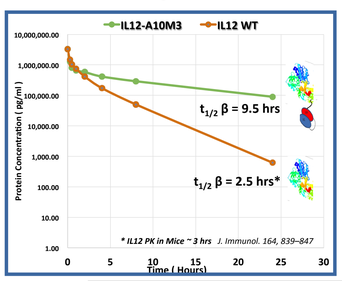
Sonnet wishes to acknowledge Drs. Olesia Buiakova & Yan Xu (InVivoTek, NJ) for their excellent work involving all animal studies,

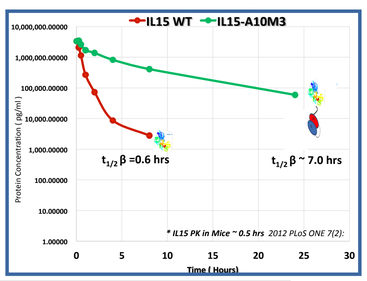
Figure 11

PK Half-Life $T^{1/2}$ of ABD Constructs IL12 & IL15

The aim of this study is to demonstrate in mice, the pharmacokinetic (PK) behavior of naked IL12 & IL15 compared to the same fusion proteins linked to albumin binding domain (ABD) IL12-ABD & IL15-ABD.

Method: 8 mice C57B/ TP, Age 9.5 weeks dose IV, sacrificed @ 5, 15, 30 mins, 1, 2, 4, 8, 24 & 48 hrs. Serum tested by ELISA

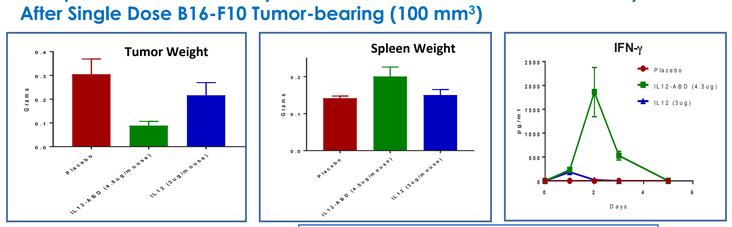




Fusion to ABD increased the plasma half-life of IL12 > 4x and IL15 >10X
IL12 MW = 70 kd vs IL15 MW=13 kd

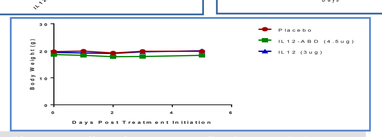
Figure 4

Comparison of Pharmacodynamic Effects of IL12-ABD & IL12 @ 5 Days After Single Dose B16-F10 Tumor-bearing (100 mm^3)



Equal molar doses of IL12 = 3ug

IL12-ABD 4.5ug = IL12 3ug



IL12-ABD demonstrated a greater suppression of tumor growth and a corresponding increase in immune activation as shown by the increase in spleen weight and a transient increase in serum IFN without an effect on mouse bodyweight compared to the molar equivalent dose of recombinant IL12.

Figure 8

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