B7-H4-specific antibodies were isolated from full length human IgG1 naïve antibody libraries using an in vitro yeast based platform. After multiple rounds of selection the resulting IgGs were sequenced and unique antibodies produced and evaluated for binding activity to recombinant B7-H4 ectodomain and epitope binning by Surface Plasmon Resonance (SPR), and for target specific cell binding by flow cytometry.

**T Cell Checkpoint Blockade Activity**

**Figure 1.** Identification B7-H4 Monoclonal Antibodies with T Cell Checkpoint Blockade Activity

1. Measure improvement in T cell proliferation by Edu incorporation (FACS)
2. Measure improvement in IFN-g production (ELISA)

**Figure 2.** FPA150 is a High Affinity, Epitope Bin 2/3 Derived mAb that Binds the B7-H4 IgV Ectodomain and is Fully Species Cross-Reactive

**Figure 3.** FPA150 is an Afucosylated huIgG1-based mAb that Demonstrates Potent T Cell Checkpoint Blockade and ADCC Activity in Vitro

**Figure 4.** FPA150 Demonstrates Potent Dose-Dependent Anti-Tumor Activity In Vivo in a CT26-mouseB7-H4/mouseB7-H3 Tumor Model

**Summary and Conclusions**

- We successfully generated a therapeutic candidate B7-H4 antibody (FPA150) which possesses both T cell immune checkpoint blockade and ADCC activity in vitro and demonstrates significant dose-dependent anti-tumor activity in vivo.
- B7-H4 antibodies with T cell checkpoint blockade activity bind and block an evolutionarily conserved functional epitope within the B7-H4 IgV ectodomain.
- In rat and cynomolgus monkey pilot PK and toxicity studies, FPA150 demonstrates a typical antibody PK profile without any toxicity.
- These data suggest that the B7-H4 mAb FPA150, which possesses T cell checkpoint and ADCC activity, has the potential to be an effective therapeutic by improving anti-tumor immune responses in cancer patients.
- IND-enabling studies are ongoing.
- Corresponding author email address: charles.kaplan@fiveprime.com

Charles D. Kaplan1, Derrick Houser2, Felicia Kemp1, Neils Nielsen4, Amy W. Hsu1, Katrina LeGris1, Gloria Brattich1, Hong Xiang1, Ago Ahene1, Ursula Jeffry1, David Bellovin1, Luis Borges1

1Five Prime Therapeutics, Two Corporate Drive, South San Francisco, California 94080 and 2Adimab, Seven Lucent Drive, Lebanon, New Hampshire, 03766

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Introduction

B7-H4 (B7, B7-S1 and VTCN1) is a type I transmembrane protein comprised of both IgV and IgC ectodomains. While B7-H4 expression in healthy tissues is relatively limited at the protein level, B7-H4 is expressed in several solid tumors such as carcinomas of the breast, ovary and endometrium, and expression tends to correlate with patient outcome, and its role as a T cell checkpoint. Given its preferential overexpression pattern in solid tumors, its negative correlation with poor prognosis, the receptor for B7-H4 appears to be an ideal target for the development of a therapeutic antibody. Here we sought to generate B7-H4 monoclonal antibodies (mAbs) that would both block the inhibitory activity of B7-H4 against T cells as well as directly lead to the depletion of B7-H4 expressing cells via antibody-dependent cell-mediated cytotoxicity (ADCC).

**Figure 2.** T Cell Checkpoint Blockade

**Figure 3.** Checkpoint Blockade Assay

**Figure 4.** ADCC Assay

**Figure 5.** Tumor Measurements

**Figure 6.** CD4+ T cells

**Figure 7.** Total T cells

**Figure 8.** IFN-g

**Figure 9.** Bin 2 mAbs

**Figure 10.** Bin 2/3 mAbs

**Figure 11.** Bin 3/4 mAbs

**Figure 12.** Bin 4 mAbs

**Table 1.** Tumor Measurements

**Table 2.** CD4+ T cells

**Table 3.** Total T cells

**Table 4.** IFN-g