AACR 1629 An innovative approach to improve bispecific T-cell engagers for solid tumor therapy

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Abstract

T cell engagers (TCEs), mainly in the format of Bispecific T-cell Engagers (BiTE), are promising anti-cancer immunotherapies for hematological malignancies. However, solid tumors create immunosuppressive microenvironments and physical structures that hinder the effectiveness of T- cell engagers and preclude tumor infiltration by CD8$^+$ T cells. Our hypothesis for overcoming these barriers is to add components to the BiTE format that alleviate immunosuppression and promote activation and tumor infiltration of CD8$^+$ T cells. To test this hypothesis, we used our novel TRBC platform to construct HCW11-018b, a tetravalent heterodimeric TCE. The BiTE portion of HCW11-018b contains an antibody that targets human tissue factor (TF), which is overexpressed in a wide spectrum of solid tumors; an anti-CD3 single-chain antibody; and an IL-15Rα domain. The other chain of HCW11-018b comprises a dimeric soluble TGFβRII domain (i.e., TGFβ trap), TRβC1 and a soluble IL-15. The plasmids carrying the coding regions of the two fusion proteins were co-transfected into CHO cells and the fully functional TCE was purified from culture supernatant. In vitro, we found HCW11-018b induced robust, antigen-specific tumor cell killing. Increased phosphorylation of STAT5, expression of activation markers (CD69, CD25), and anti-apoptosis marker (BCL2) were observed on T cells by HCW11-018b treatments. RNAseq analysis revealed that increased expression of BCL2A1, BCL2L1 and other genes associated with T-cell effector function were specifically attributable to the IL-15 component of HCW11-018b. In SCID mice subcutaneously (s.c.) implanted human AsPC-1 pancreatic cells with human PBMCs, we demonstrated that s.c. administered HCW11-018b could infiltrate into implants and activate bystander CD8+ T cells for potent anti-tumor activities. In NSG mice bearing AsPC-1 tumors and adoptively transferred human T cells, HCW11-018b stimulated expression of CCR5 and promoted tumor infiltration of human T cells, upregulated T cell expression of CD25, NKG2D, DNAM1, Granzyme B, and IFNγ, and enhanced their cytotoxicity against cancer cells. Treatment also reduced tumor cell metastasis from the primary site. We further demonstrated that the upregulation of NKG2D and DNAM1 on HCW11-018b-activated CD8$^+$ T cells played a role in the cytotoxicity against AsPC-1 cells. In a PDX model, we further demonstrated the potency of HCW11-018b which directed hPBMCs against TF$^+$ patient-derived pancreatic cancer tissues. HCW11-018b was well tolerated in mice and non-human primates with s.c. administration. HCW11-018b is currently in IND-enabling studies for clinical development against solid tumors. In summary, we demonstrate that the addition of TGFβ-trap and IL-15 components onto BiTE using our novel TRBC platform can overcome the deficiencies of BiTE for solid tumor therapy.

An innovative approach to improve bispecific T-cell engagers

A	HCW11-018b	IL-15 Activity (EC50)	TGF-β Trapping activity (IC50)	TF Binding (KD)	CD3D/E Binding (KD)	IL-2Rβγ Binding (KD)
	TGFβRII/hTRβC1/IL-15 :αTF/IL-15RαSu/αCD3	35.61pM	55.94pM	144pM	2.22nM	2.36nM

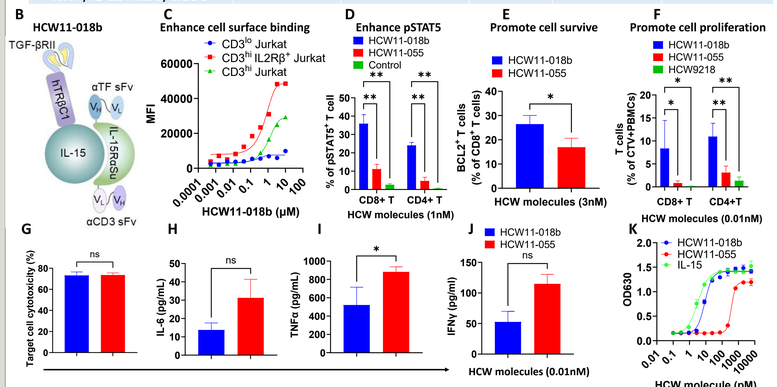

Figure 1. An innovative approach to improve bispecific T-cell engagers for solid tumor therapy. The activity of each component of HCW11-018b (A). Scheme of HCW11-018b (B). The IL-15 component of HCW11-018b contributed: to enhance cis-binding of anti-CD3 on IL-2Rβ expressed Jurkat cells (C); to promote STAT5 phosphorylation in both CD8+ T cells and CD4+ T cells (D); to promote T cell survival (E); to promote T cell proliferation (F); but not contribute to target cell cytotoxicity or cytokine release (G-J). HCW11-055 is a HCW11-18b control molecule containing a less functional IL-15 (D8N) mutant analyzed by HEK-Blue™ CD122-CD132 assay (K).

HCW11-018b exhibits target specific *in vitro* T cell activation and cytotoxicity, and *in vivo* anti-tumor activity

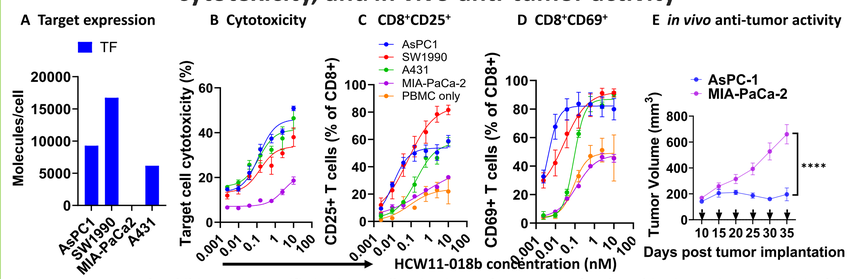

Figure 2. HCW11-018b exhibits target specific *in vitro* T cell activation and cytotoxicity (A-D), and *in vivo* anti-tumor activity (E).

HCW11-018b induces transcriptional changes in T cells

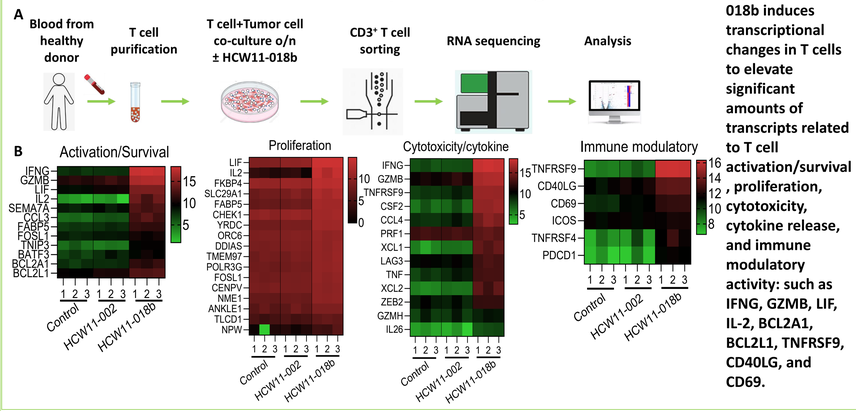

Figure 3. HCW11-018b induces transcriptional changes in T cells to elevate significant amounts of transcripts related to T cell activation/survival, proliferation, cytotoxicity, cytokine release, and immune modulatory activity: such as IFNG, GZMB, LIF, IL-2, BCL2A1, BCL2L1, TNFRSF9, CD40LG, and CD69.

HCW11-018b exhibits potent *in vivo* anti-tumor activity by promoting tumor infiltration of CD8$^+$ T cells and the subsets with higher NKG2D$^+$/Gzmb$^+$ populations.

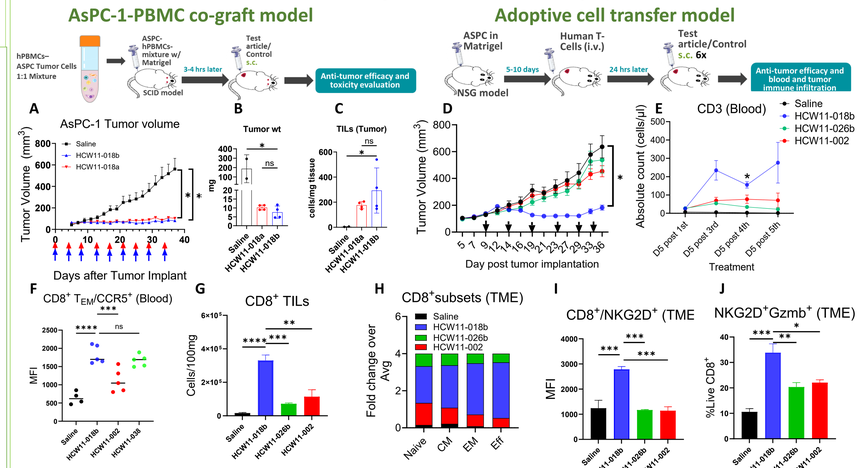

Figure 4. HCW11-018b treatment induces potent anti-tumor activity in both co-graft model (A-B) and adoptive cell transfer model (D). HCW11-018b promotes T cell infiltration and expansion in tumors in the co-graft model (C). In the adoptive cell transfer model, HCW11-018b treatment results in expansion of T cells with effector phenotype (CCR5, E-H), tumor-infiltrated CD8+ T cells expressing high NKG2D and Gzmb (I-J) and reduction of lung metastasis (K). Blocking NKG2D/DNAM-1 on human T cells *in vitro* partially inhibited target cell killing (L). HCW11-018a, HCW11-026b, HCW11-038, and HCW11-002, are HCW11-018b control molecules containing an IL-7 to replace the TGFβ trap, an anti-MSLN scFv to replace anti-TF scFv, an anti-MSLN scFv to replace the TGFβ trap, or two TGFβ traps but no bispecific T-cell engagers, respectively.

HCW11-018b exhibits potent anti-tumor activity in a tissue factor expressing patient-derived xenograft (PDX) model of cancer

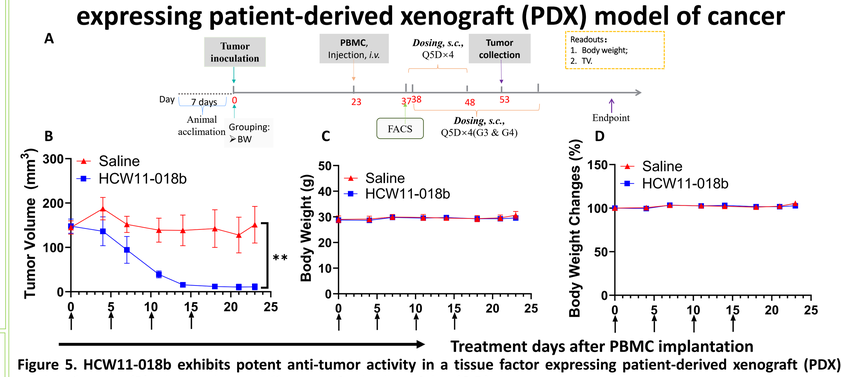

Figure 5. HCW11-018b exhibits potent anti-tumor activity in a tissue factor expressing patient-derived xenograft (PDX) model of cancer (B) whereas has no effect on mice body weights (C-D).

Safety evaluation of HCW11-018b in a non-human primate model: investigating immune cell and cytokine responses and pharmacokinetic characteristics

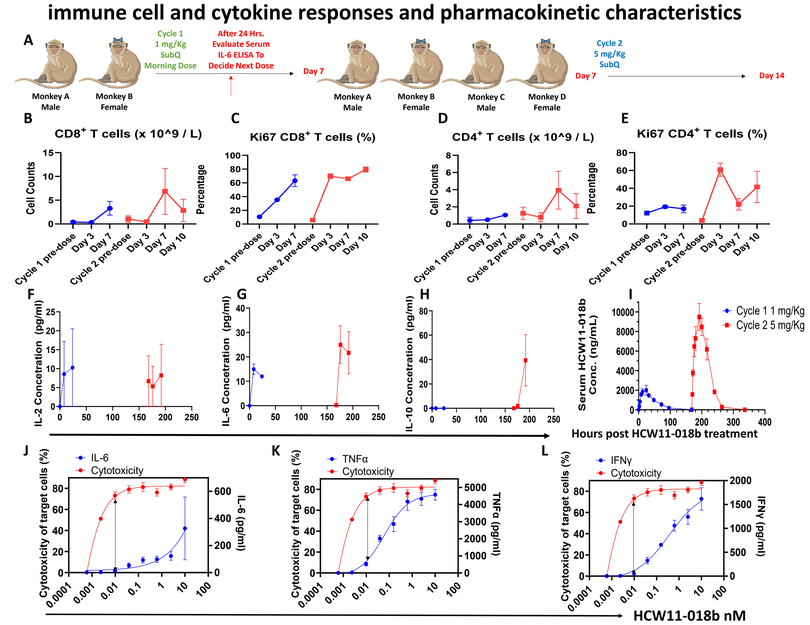

Figure 6. Safety evaluation of HCW11-018b in a non-human primate model: HCW11-018b treatment stimulated significant proliferation and expansion of CD8$^+$ T cells and modest expansion of CD4$^+$ T cells (B-E) with little or no induction of pro-inflammatory cytokines (F-H). No acute toxicity was observed at the 1 or 5 mg/kg dose levels. The mean half-life of HCW11-018b in non-human primate was 19.87 hours and C_{max}=2.005 μg/mL (I). *In vitro* comparison of target cell cytotoxicity and cytokine release suggests that HCW11-018b at 0.01 nM may provide an optimal therapeutic window with high anti-tumor efficacy and low cytokine release (J-L).

Conclusion

- The fusion with TGFR-TRBC1-IL15 made the anti-TF/anti-CD3 T cell engager more stable with a serum half life of about 20 hours in non-human primates after 1 mg/kg subcutaneous administration.
- The IL-15 component of HCW11-018b contributed to enhanced cis-binding of the anti-CD3 component to T cells, increased phosphorylation of STAT5, and promoted cell survival and proliferation.
- The IL-15 component of HCW11-018b did not contribute to target cell cytotoxicity or cytokine release.
- HCW11-018b was very effective at promoting T cell activity against tumor cells both *in vitro* and in *vivo*.
- At a potential therapeutic dose (0.01nM), HCW11-018b induce potent anti-tumor T cell cytotoxicity with low cytokine release, especially IL-6.

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