

A novel multi-functional bispecific T-cell engager molecule for cancer therapy

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Abstract

Pancreatic cancer (PC) is highly aggressive, with a median survival time of less than 6 months and a 5-year overall survival rate of around 13% (1, 2). Current treatments are largely ineffective, representing a significant unmet medical need. To address this, we used our novel TRBC (T-cell receptor β -chain constant region 1) platform technology to develop a multi-functional bispecific T-cell engager (TCE) targeting human tissue factor (TF), which is overexpressed in pancreatic cancer. This TCE immunotherapeutic, designated as HCW11-018b, comprises anti-TF/anti-CD3 single chain antibody domains as well as soluble TGF β R2 (i.e., TGF β trap) and soluble IL-15/IL-15R α Su complex. *In vitro* experiments demonstrated all components of HCW11-018b expressed and functioned well. In addition to the profound cytotoxicity to target cells as traditional TCE, this novel TCE activated pSTAT5 pathway, increased BCL2, promoted T-cell proliferation and division, made T-cell capable of sustained cytotoxicity to target cells. Both *in vitro* and *in vivo* experiments demonstrated T-cell activation and anti-tumor activity by HCW11-018b were target specific. In the co-grafting model, human PBMC were administered with AsPC-1 cells (E:T, 1:1, s.c.) into SCID mice followed by treatment with HCW11-018b (1mg/kg, s.c.)(3). Treatments were administered every 4 days. TCE effectively engaged human T cells, to promote significant tumor regression and complete responses compared to controls. In ACT experiments, after s.c. AsPC-1 tumors reached 50-100mm³, mice received intravenous injection of purified human T cells followed by HCW11-018b (1mg/kg, s.c.) the following day. Treatments were administered every 5 days. TCE effectively engaged adoptively transferred human T cells, induced tumor immune infiltration of cytotoxic (Granzyme B⁺, NKG2D⁺) CD8⁺ T cells to promote significant tumor regression and complete responses compared to controls. The anti-tumor efficacy of HCW11-018b was further evaluated in a patient-derived xenograft (PDX) mouse pancreatic cancer model. In PDX model, after s.c. PDX cancer reached 50-100mm³, mice received intravenous injection of human PBMCs followed by HCW11-018b (1mg/kg, s.c.). Treatments were administered every 5 days. TCE effectively engaged adoptively transferred human T cells, to promote significant tumor regression and complete responses compared to controls. Additionally, HCW11-018b was well tolerated in tested mouse models (absence of lethargy, hunching, and body weight loss). In non-human primates, HCW11-018b was well tolerated even in a higher dose (5mg/kg). The inflammatory cytokine levels were very low, and no adverse effects were observed. HCW11-018b is currently in an IND-enabling process for future clinical development against PC. Altogether, these data demonstrate that: (a) our TRBC-based TCE can induce potent anti-tumor activity against 'difficult-to-treat' pancreatic tumors, and (b) the versatility of the TRBC platform can be used to create next-generation multi-specific T-cell engagers against solid tumors.

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

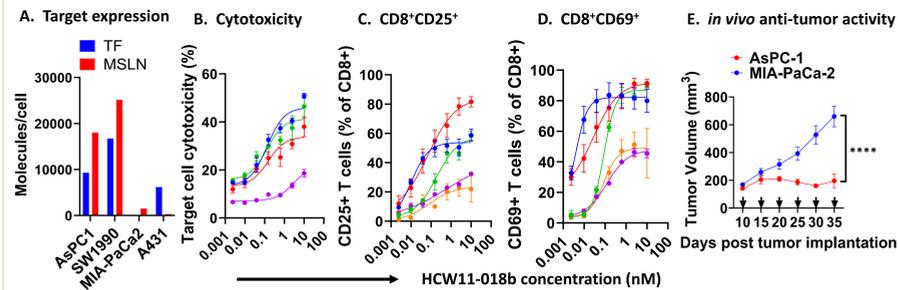


Figure 2. HCW11-018b exhibits target specific *in vitro* T cell activation and cytotoxicity (A-D), and *in vivo* anti-tumor activity (E).

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

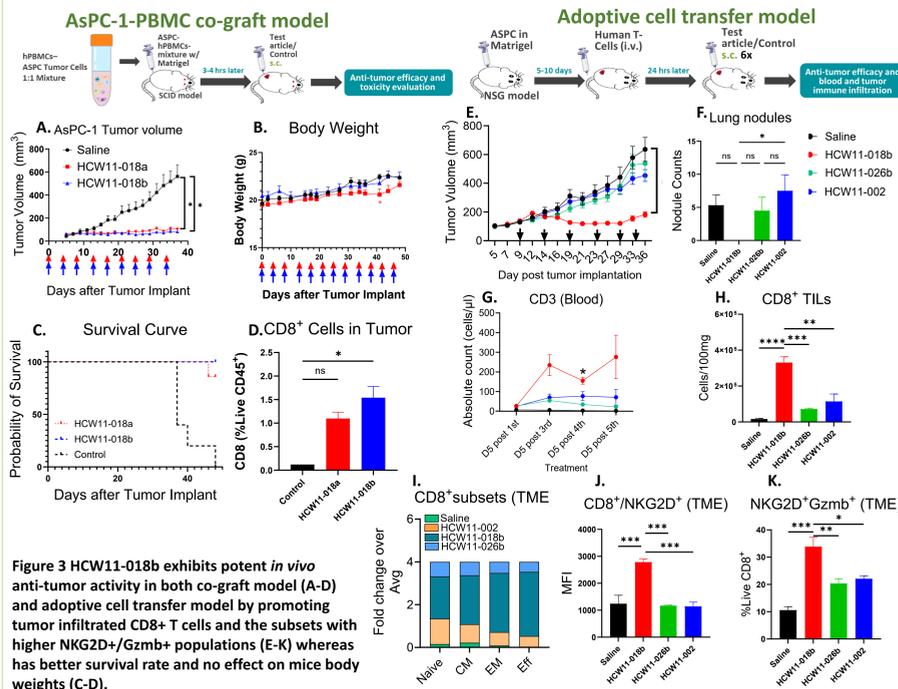


Figure 3 HCW11-018b exhibits potent *in vivo* anti-tumor activity in both co-graft model (A-D) and adoptive cell transfer model by promoting tumor infiltrated CD8⁺ T cells and the subsets with higher NKG2D⁺/Gzmb⁺ populations (E-K) whereas has better survival rate and no effect on mice body weights (C-D).

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

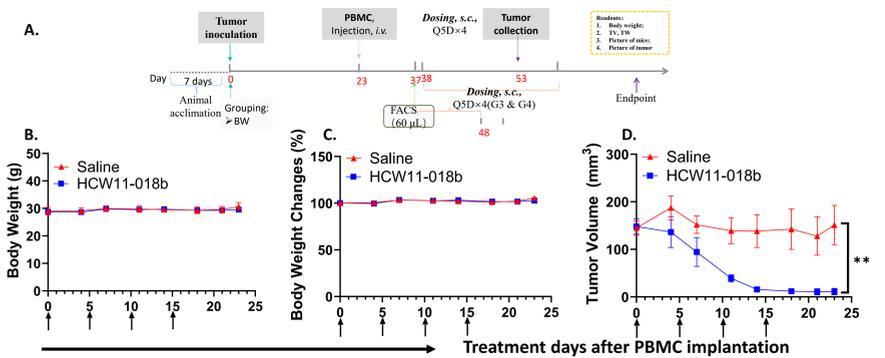


Figure 5. HCW11-018b exhibits potent anti-tumor activity in a tissue factor expressing patient-derived xenograft (PDX) model of cancer (D) whereas has no effect on mice body weights (B-C).

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

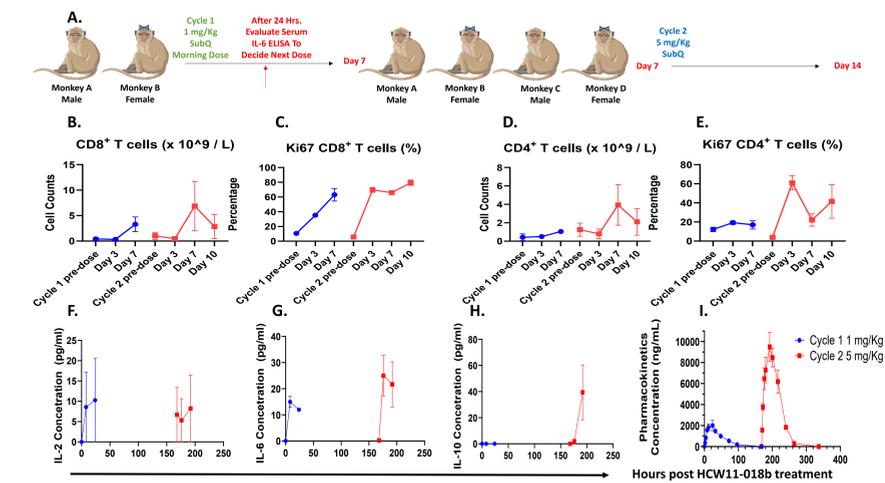


Figure 6. Safety evaluation of HCW11-018b in a non-human primate model: HCW11-018b treatment stimulated significant proliferation and expansion of CD8⁺ T cells and modest expansion of CD4⁺ T cells (B-E) with little or no induction of pro-inflammatory cytokines (F-H). No acute toxicity was observed at 1 or 5 mg/kg dose levels. The mean half-life of HCW11-018b was 19.87 hours (I).

Novel bispecific T cell engagers with enhanced functions

Protein	HCW Molecules	IL-7 Activity (EC50)	IL-15 Activity (EC50)	TGF- β Trap Activity (IC50)	TF Binding (Kd)	CD3D/E Binding (Kd)	MSLN Binding (Kd)
IL-7/hTRBC1/IL-15: α TF/IL-15R α Su/ α CD3	11-018a	8.66pM	16.19pM	55.94pM	4.817nM	4.088nM	
TGF β R2/hTRBC1/IL-15: α TF/IL-15R α Su/ α CD3	11-018b		44.24pM	100.7pM	3.739nM	4.006nM	
TGF β R2/hTRBC1/IL-15: α Mesothelin (MSLN)/IL-15R α Su/ α CD3	11-026b		64.29pM			5.103nM	29.19nM
TGF β R2/hTRBC1/IL-15:TGF β R2/IL-15R α Su	11-002		72.45pM	13.24pM			

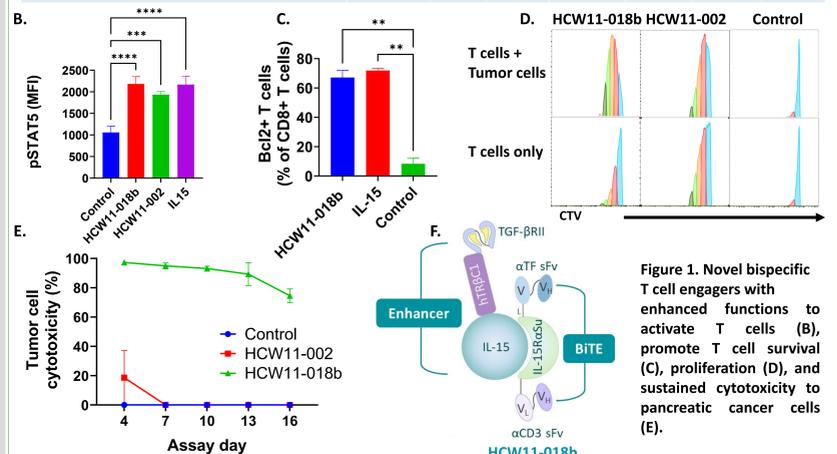


Figure 1. Novel bispecific T cell engagers with enhanced functions to activate T cells (B), promote T cell survival (C), proliferation (D), and sustained cytotoxicity to pancreatic cancer cells (E).

HCW11-018b induces transcriptional changes in T cells

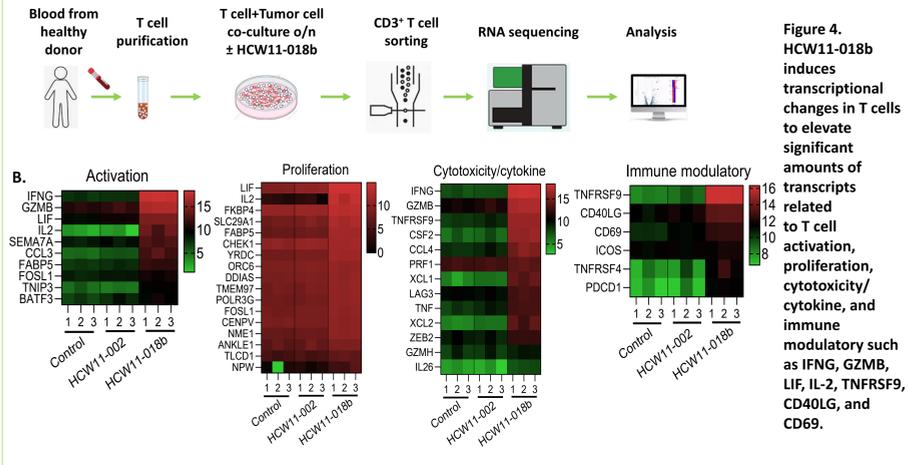
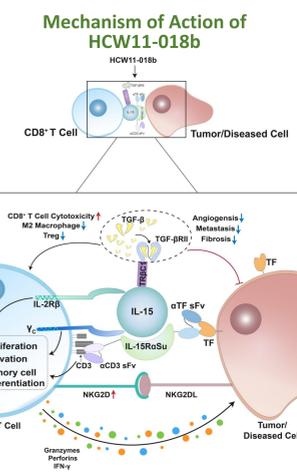


Figure 4. HCW11-018b induces transcriptional changes in T cells to elevate significant amounts of transcripts related to T cell activation, proliferation, cytotoxicity/cytokine, and immune modulatory such as IFNG, GZMB, IL2, TNFRSF9, CD40LG, and CD69.

HCW T-Cell Engagers (TCEs) superior efficacy over conventional bispecific TCEs

HCW's TCEs Unique Features	HCW's TCEs Activities	HCW's TCEs	Conventional Bi-Specific TCEs
Solid Tumors	Directs TCE to specifically target solid tumor cells with proprietary sFvs, enhancing specificity, reducing off-tumor toxicity, focusing the immune response at the tumor site, and minimizing systemic immune activation.	Novel non-Fc-based approach to minimize risks and to increase serum half-life	Majority utilizes Fc to extend the half-life
αCD3: Activating T Cells	Binds CD3 on T cells to redirect them toward tumor cell killing, activating polyclonal T cells irrespective of TCR specificity, enabling immune synapse formation, and inducing cytotoxicity even without tumor-specific T cells.	Available	Available
IL-15: Improving Proliferation and Survival of T Cells	Promotes proliferation and survival of T and NK cells, enhances effector and memory T cell expansion and persistence, supports long-term antitumor immunity, and is less likely to expand regulatory T cells than IL-2.	Available	Not Available
TGF-βR2: Anti-Tumor Mediated Immunosuppression	Blocks TGF- β signaling by sequestration or decoy receptor action, overcoming tumor-mediated immunosuppression, restoring T cell effector function, and enhancing cytotoxic T cell infiltration and activity in tumors.	Available	Not Available
Promoting Immune Cells Infiltration Into Solid Tumors	Efficacy Improvement.	High Degree	Unknown
Molecule Infiltration Into Solid Tumors	Efficacy Improvement.	High Degree	Unknown
Subcutaneous Administration	Side Effect Management: No CRS and overt AE in NHPs at high dose levels.	Lower Risk	High Risk
Manufacturing Process	Streamline at a lower cost	Similar to therapeutic Ab	Tedious



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