PHST001, a humanized anti-CD24 antibody, induces phagocytosis of ovarian tumor cells in vitro and inhibits their growth in vivo

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Background

- CD24 (Cluster of differentiation 24) is a small, highly glycosylated tumor antigen that acts as an important macrophage "don't eat me" signal[1]
  - It interacts with the macrophage receptor Siglec-10 (Sialic-acid-binding Ig-like lectin 10) to protect cancer cells from being engulfed by tumor-associated macrophages[1]
- CD24 is highly expressed on a subset of cancers; in some tumor types, it is upregulated greater than 100-fold compared to paired normal tissues. This overexpression is most frequent in solid tumors, including ovarian cancer[1,2]
- PHST001, a humanized IgG4 anti-CD24 monoclonal antibody, blocks the interaction of CD24 and Siglec-10 to induce phagocytosis of cancer cells
- **The purpose of this project is to assess efficacy of PHST001 against ovarian cancer in vitro and in vivo**

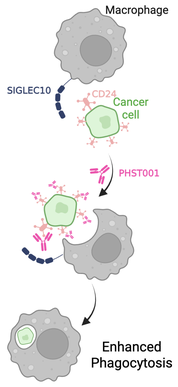
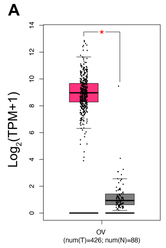


Figure 1: Ovarian tumors express high levels of *CD24* mRNA and present frequent *CD24* copy number gain



B

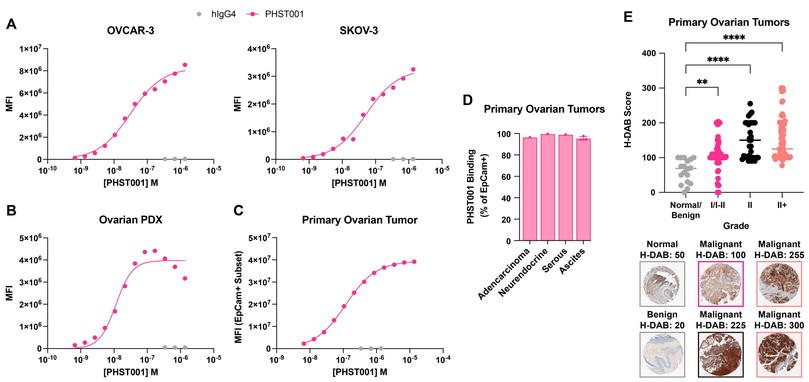
CD24 copy number gain is highly enriched in high grades of ovarian cancer

	CD24 Copy Number Gain	
Tumor Grade by Histology (n)	Number of events	Prevalence, %
GB (2)	0	0.0
G1 (5)	0	0.0
G2 (53)	25	32.1
G3-4 (280)	123	30.5
GX (5)	2	28.6
NA (79)	38	32.5
Total (424)	**188**	**30.7**

GX: grade cannot be evaluated; GB: low malignant potential; G1: grade 1; G2: grade 2; G3-4: grades 3 and 4. *CD24* amplification (CD24 amp) is defined as copy number > 2.14.

A. *CD24* mRNA expression from the Cancer Genome Atlas (TCGA; 426 samples) and Genotype-Tissue Expression (GTEx; 88 samples) datasets. Ovarian cancer samples (pink boxplot) express higher levels of *CD24* mRNA than normal ovarian samples (gray boxplot). P<0.05. **B.** *CD24* copy number alteration data from TCGA. Copy number gain is defined as copy number >2.14.

Figure 2: PHST001 binds robustly to ovarian cancer cell lines and primary ovarian cancer samples

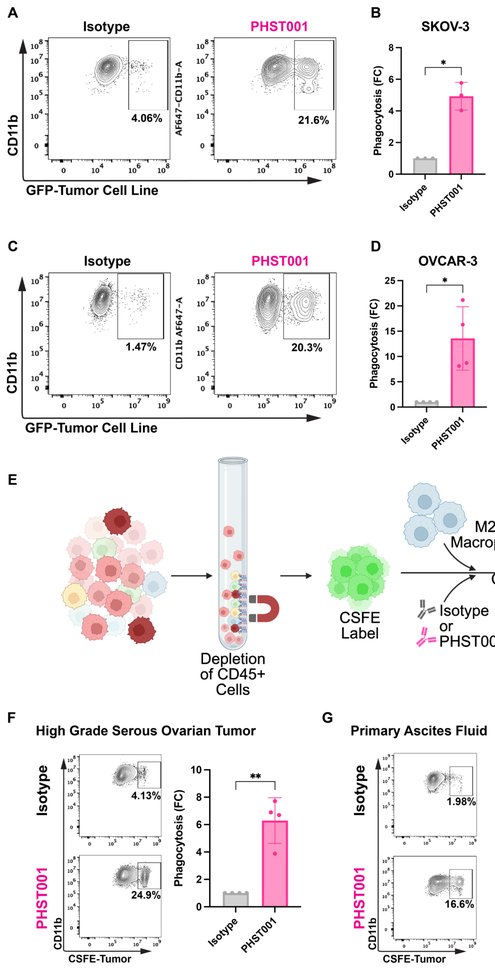


A. (left) Binding curve fit of OVCAR-3 cells with PHST001. **(right)** Binding curve fit of SKOV-3 cells with PHST001. **B.** Binding curve fit of ovarian PDX cells with PHST001. **C.** Binding curve fit of EpCam+ tumor cells from a primary ovarian cancer sample with PHST001. **D.** PHST001 at 50μg/ml binding to EpCam+ tumor cells from primary ovarian tumors. PHST001 binds to ~100% tumor cells from different subtypes of ovarian cancer. **E. (top)** IHC of tumor microarrays stained with PHST001-mIgG1k separated by grade and quantified by pathologist H-Score. (n=18 normal/benign, n=191 tumor cores). **P<0.01, ****P<0.0001. **(bottom)** representative images of IHC staining on ovarian tissue cores.

Conclusions

- CD24 is overexpressed in ovarian cancers
- Ovarian cancers present frequent *CD24* copy number gain
- PHST001 binds to tumor cells present in primary ovarian tumors and induces phagocytosis of multiple subtypes of ovarian cancer cells in vitro
- PHST001 has significant in vivo efficacy in ovarian cancer models both as monotherapy and in combination with chemotherapy or an antibody-drug conjugate
- A Phase 1 clinical study (NCT06840886) investigating the safety and tolerability of PHST001 in adult patients with relapsed/refractory solid tumors, including ovarian cancers, is ongoing

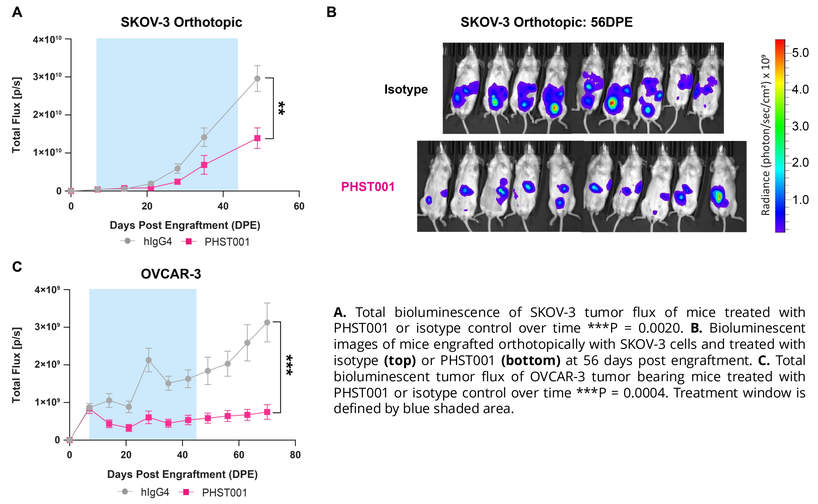
Figure 3: PHST001 induces phagocytosis of ovarian cell lines and primary ovarian tumors in vitro



A. Representative FACS plots for SKOV-3 cells treated with Isotype control **(left)** or PHST001 **(right)**. **B.** Phagocytosis as fold change (FC) by M2 macrophages of SKOV-3 cells treated with PHST001 relative to isotype. *P<0.05. **C.** Representative FACS plots for OVCAR-3 cells treated with Isotype **(left)** or PHST001 **(right)**. **D.** Phagocytosis as fold change (FC) by M2 macrophages of OVCAR-3 cells treated with PHST001 relative to isotype. *P<0.05.

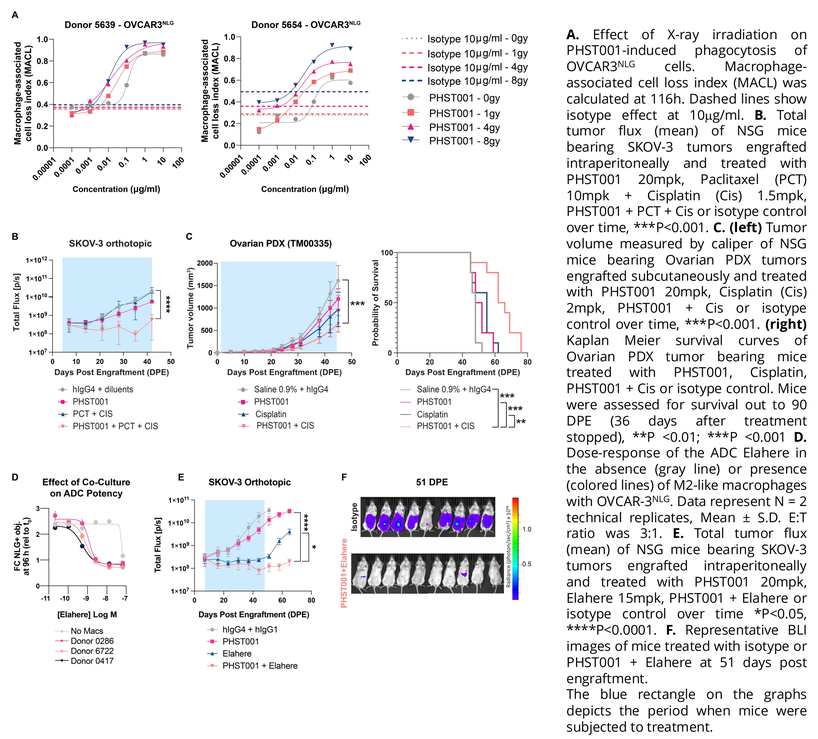
E. Schematic depicting workflow for processing of primary human tumors for flow cytometry-based phagocytosis assay. **F. (left)** Representative FACS plots of phagocytosis assay utilizing isolated EpCam+ tumor cells treated with isotype **(top)** or PHST001 **(bottom)**. **(right)** Quantification of phagocytosis assay. **P<0.01. **G.** Representative FACS plots of phagocytosis assay utilizing isolated EpCam+ tumor cells derived from patient ascites treated with isotype **(top)** or PHST001 **(bottom)**.

Figure 4: PHST001 is efficacious as monotherapy against ovarian cancer models in vivo



A. Total bioluminescence of SKOV-3 tumor flux of mice treated with PHST001 or isotype control over time ***P = 0.0020. **B.** Bioluminescent images of mice engrafted orthotopically with SKOV-3 cells and treated with isotype **(top)** or PHST001 **(bottom)** at 56 days post engraftment. **C.** Total bioluminescent tumor flux of OVCAR-3 tumor bearing mice treated with PHST001 or isotype control over time ***P = 0.0004. Treatment window is defined by blue shaded area.

Figure 5: PHST001 combines with standard of care in vitro and in vivo



A. Effect of X-ray irradiation on PHST001-induced phagocytosis of OVCAR3[NLG] cells. Macrophage-associated cell loss index (MACL) was calculated at 116h. Dashed lines show isotype effect at 10μg/ml. **B.** Total tumor flux (mean) of NSG mice bearing SKOV-3 tumors engrafted intraperitoneally and treated with PHST001 20mpk, Paclitaxel (PCT) 10mpk + Cisplatin (Cis) 1.5mpk, PHST001 + PCT + Cis or isotype control over time, ***P<0.001. **C. (left)** Tumor volume measured by caliper of NSG mice bearing Ovarian PDX tumors engrafted subcutaneously and treated with PHST001 20mpk, Cisplatin (Cis) 2mpk, PHST001 + Cis or isotype control over time, ***P<0.001. **(right)** Kaplan Meier survival curves of Ovarian PDX tumor bearing mice treated with PHST001, Cisplatin, PHST001 + Cis or isotype control. Mice were assessed for survival out to 90 DPE (36 days after treatment stopped), **P <0.01; ***P <0.001 **D.** Dose-response of the ADC Elahere in the absence (gray line) or presence (colored lines) of M2-like macrophages with OVCAR-3[NLG]. Data represent N = 2 technical replicates, Mean ± S.D. E:T ratio was 3:1. **E.** Total tumor flux (mean) of NSG mice bearing SKOV-3 tumors engrafted intraperitoneally and treated with PHST001 20mpk, Elahere 15mpk, PHST001 + Elahere or isotype control over time *P<0.05, ****P<0.0001. **F.** Representative BLI images of mice treated with isotype or PHST001 + Elahere at 51 days post engraftment.

The blue rectangle on the graphs depicts the period when mice were subjected to treatment.

Abbreviations: ADC, antibody-drug conjugate; BLI, bioluminescence imaging; DAB, diaminobenzidine; FACS, fluorescence-activated cell sorting; FC, fold-change; GFP, green fluorescent protein; IHC, immuno-histochemistry; MFI, mean fluorescence intensity; NLG, nuclear localized GFP.

References: 1. Barkal AA, *et al*. CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*. 2019;572(7769):392-396. doi:10.1038/s41586-019-1456-0; 2. Tang Z, *et al*. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res*. 2017;45(W1):W98-W102.