PHST001 combination with standard-of-care chemotherapy enhances macrophage-mediated elimination of tumor cells

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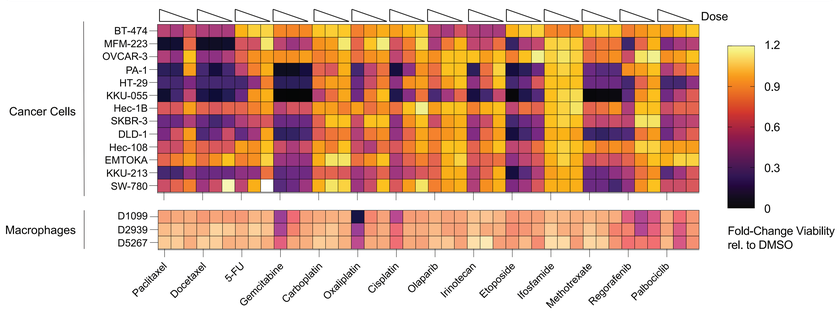
Background

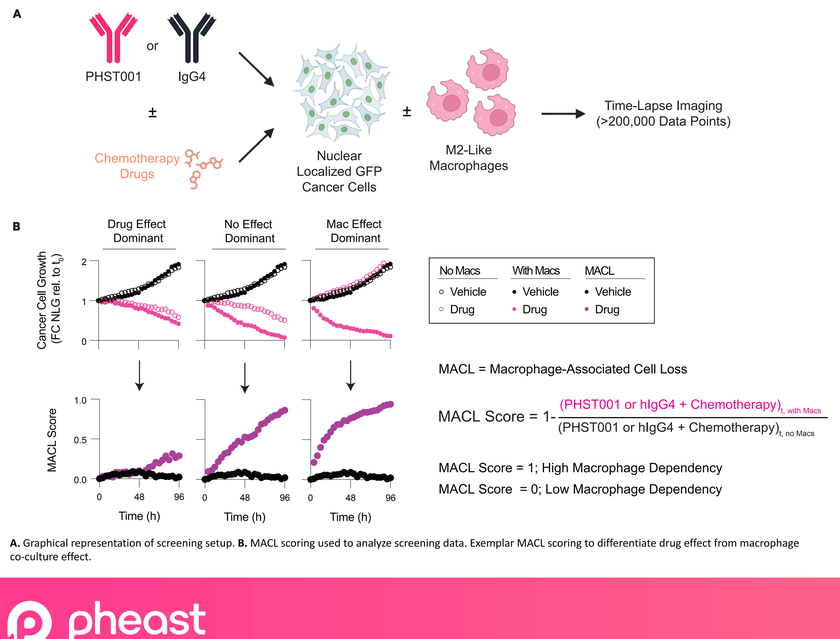
- The balance between “eat me” and “don’t eat me” signals contributes to the regulation of macrophage phagocytosis[1]
- CD24 (Cluster of differentiation 24) is a “don’t eat me” signal that is often over-expressed on cancer cells and inhibits macrophage phagocytosis via interaction with the ITIM-containing Siglec-10 (sialic-acid-binding Ig-like lectin-10) receptor[2]
- PHST001 is a clinical-stage monoclonal antibody that targets CD24 and blocks its interaction with Siglec-10[2]
- Chemotherapy induces cell stress and can upregulate “eat me” signals on cancer cells[3]
- We hypothesize that PHST001 may deepen tumor cell killing when combined with chemotherapy agents, through concomitant blockade of a “don’t eat me” signal in the context of increased “eat me” signals on target tumor cells

Figure 1. Macrophages were more resistant to chemotherapy than 13 cancer cell lines representing six cancer types[a]



[a]13 cancer cell lines or M2-like (TGF-β/IL-10 stimulated) macrophages derived from the PBMCs of three healthy donors were treated with 14 common chemotherapy agents in a 3-point, 4-fold dose-response. Data represent fold-change in growth relative to DMSO at 72 h post treatment.

Figure 2. An *in-vitro* screen identified potential chemotherapy combination partners for PHST001

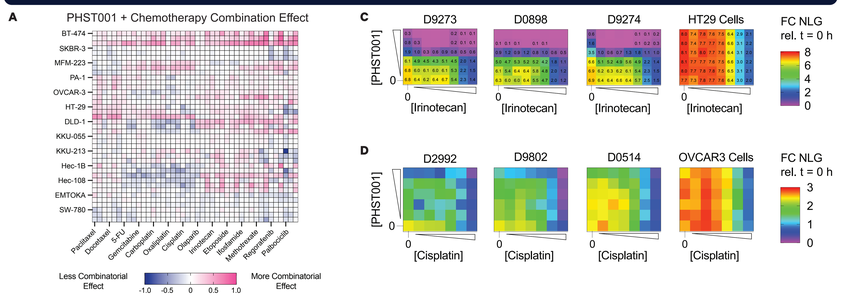


A. Graphical representation of screening setup. **B.** MACL scoring used to analyze screening data. Exemplar MACL scoring to differentiate drug effect from macrophage co-culture effect.

Conclusions

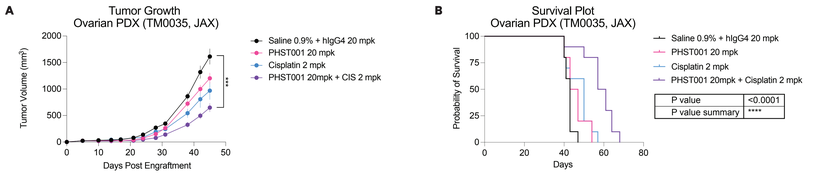
- Unlike cancer cell lines, macrophages display broad resistance to common chemotherapy drugs
- Across cancer cell lines, PHST001 often demonstrated strong synergy with chemotherapy agents, including taxanes, topoisomerase inhibitors, antimetabolites, and platins
- PHST001 in combination with cisplatin reduced tumor growth and increased survival in an aggressive, ovarian patient-derived xenograft model
- Expression of the phosphatidylserine “eat me” signal increased in response to treatment with some chemotherapies, including taxanes, and correlated with increased macrophage phagocytic activity
- Macrophages activated by gemcitabine demonstrated increased phagocytosis of PHST001-coated cancer cells in the absence of additional gemcitabine, suggesting that synergy between these agents can occur independently of direct exposure of cancer cells to chemotherapy

Figure 3. PHST001 in combination with chemotherapy is more effective than either agent alone



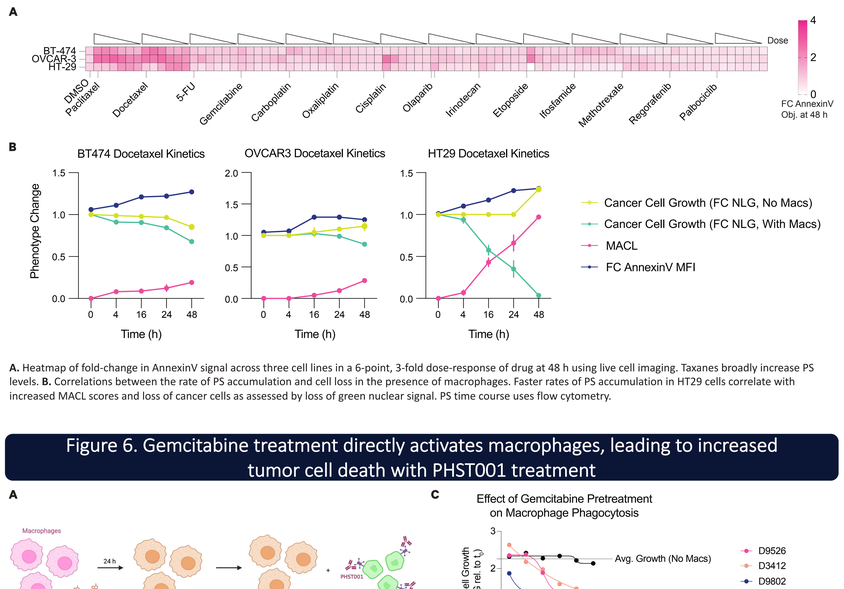
A. Minimum distance analysis between PHST001 + chemotherapy combination relative to PHST001 alone, chemotherapy alone, or IgG4 + chemotherapy. Greater distance (pink) denotes more combinatorial effect. Each cell line was tested with three unique M2-like (TGF-β/IL-10 stimulated) macrophage lines derived from healthy volunteer PBMCs. **B.** Select chemotherapy agents with PHST001 or IgG4 across cell lines showing strong combinatorial effect. **C.** Two-way dose-response between irinotecan and PHST001 in HT29 cells, confirming hits from the primary screen. **D.** Two-way dose-response between cisplatin and PHST001 in OVCAR3 cells. Combinatorial effects in OVCAR3 cells were universally weak; however, upon increasing the dose of PHST001 we achieved combinatorial effect with cisplatin in the platin-resistant cell line OVCAR3. **E.** Kinetic growth curves extracted from two-way dose-response experiments in HT29 and OVCAR3 cells.

Figure 4. PHST001 in combination with cisplatin reduces growth of an aggressive ovarian patient-derived xenograft (PDX) model



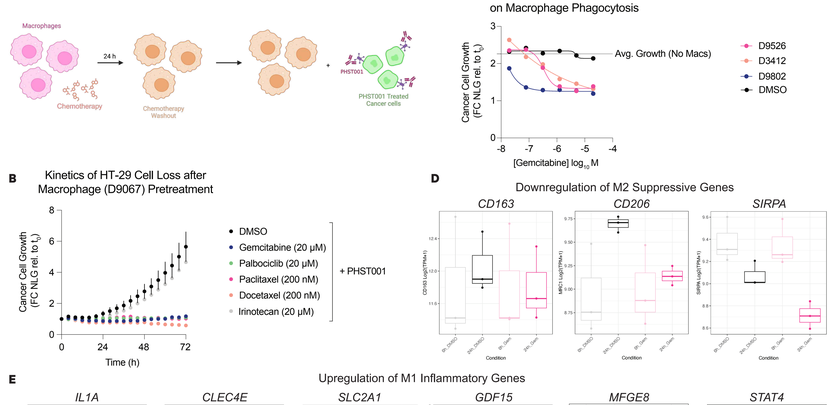
PHST001 was dosed at 20 mpk three times weekly for 47 days; cisplatin was dosed at 2 mpk once weekly for a total of five doses. Only the PHST001 and cisplatin combination arm showed a significant reduction in **A.** tumor burden and **B.** survival benefit.

Figure 5. Increased “eat me” phosphatidylserine presentation after treatment with some chemotherapies correlates with increased macrophage phagocytosis



A. Heatmap of fold-change in AnnexinV signal across three cell lines in a 6-point, 3-fold dose-response of drug at 48 h using live cell imaging. Taxanes broadly increase PS levels. **B.** Correlations between the rate of PS accumulation and cell loss in the presence of macrophages. Faster rates of PS accumulation in HT29 cells correlate with increased MACL scores and loss of cancer cells as assessed by loss of green nuclear signal. PS time course uses flow cytometry.

Figure 6. Gemcitabine treatment directly activates macrophages, leading to increased tumor cell death with PHST001 treatment



A. Schematic of chemotherapy screen on macrophage phagocytosis independent of chemotherapy effect on cancer cells. **B.** Cancer cell growth over time of HT29 cells treated with PHST001 and macrophages treated with chemotherapy 24 h prior to coculture. Gemcitabine, palbociclib, paclitaxel, and docetaxel increase macrophage killing of target cells. **C.** Dose-response of gemcitabine treatment of macrophages only, followed by treatment with PHST001-coated cancer cells. **D.** RNA sequencing reveals that gemcitabine treatment of macrophages downregulates genes associated with M2 suppressive macrophages including *CD163*, *MRC1* (*CD206*), and *SIRPA* with a (**E**) concomitant increase in M1 inflammatory genes, including *IL1A*, *CLEC4E*, *SLC2A1*, GDF15, *MFGE8*, and *STAT4*. For each boxplot: center = median, box = interquartile range, whiskers = 5th to 95th percentile

Abbreviations: Avg. average; CD, Cluster of differentiation; CLEC4E, C-type lectin domain family 4 member E; DMSO, dimethyl sulfoxide; DPE, days post engraftment; FC NLG, fold-change nuclear-localized green; 5-FU, fluorouracil; GDF15, growth differentiation factor 15; GEM, gemcitabine; GFP, green fluorescent protein; h, hour; IgG, immunoglobulin G; IL, interleukin; ITIM, immunoreceptor tyrosine-based inhibitory motif; Mac, macrophage; MACL, macrophage-associated cell loss; MFGE8, milk fat globule-EGF factor 8; MFI, mean fluorescence intensity; mpk, mg per kg; MRC1, mannose receptor C-type 1; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; PDX, patient-derived xenograft; PS, phosphatidylserine; rel, relative; Siglec-10, sialic-acid-binding Ig-like lectin-10; SIRPA, signal-regulatory protein alpha; SLC2A1, solute carrier family 2 member 1; STAT4, signal transducer and activator of transcription 4; t_0, time zero; TGF-β, transforming growth factor beta.

References: 1. Kelley SM, Ravichandran KS. Putting the brakes on phagocytosis: “don’t-eat-me” signaling in physiology and disease. *EMBO Rep.* 2021;22(6):e52564; 2. Barkal AA, *et al.* CD24 signalling through macrophage Siglec-10 is a target for cancer immunotherapy. *Nature*. 2019;572(7769):392-396; 3. Merlano MC, *et al.* How chemotherapy affects the tumor immune microenvironment: a narrative review. *Biomedicines*. 2022;10(8):1822.

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