The anti-CD47 antibody SRF231 increases anti-tumor activity of standard of care chemotherapy in platinum-resistant PDX models of ovarian cancer

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Background

CD47 is a type I integral membrane protein expressed on multiple human tumors, including ovarian cancer, and modulates cell processes such as cell migration, adhesion, T-cell function, and cell death via interaction with multiple ligands. Interaction of CD47 with SIRPα expressed on myeloid cells results in an inhibitory "don't eat me" signal that prevents phagocytosis of CD47-expressing cancer cells. Enhancement of the anti-tumor activity of chemotherapy has also been reported with CD47 antagonists. We investigated the effects of combining SRF231, an investigational fully human IgG4 anti-CD47 antibody, with chemotherapy in models of human ovarian cancer.

Methods

- Expression of CD47 in 8 established platinum-resistant PDX models of ovarian cancer was measured by immunohistochemistry with the anti-hCD47 antibody SP279.
- SRF231-mediated phagocytosis of ovarian cancer cell lines was assessed using a macrophage coculture system.
- *In vitro* tumor cell death in the presence of immobilized SRF231 with either doxorubicin or platinum was assessed by an Annexin V assay.
- The activity of SRF231 combined with doxorubicin *in vivo* was compared to isotype control, SRF231, or doxorubicin monotherapy in an ovarian cancer subcutaneous xenograft model, OVCAR3.
- Additionally, the activity of SRF231 combined with platinum was compared to isotype control, SRF231, or platinum monotherapy in two luciferase-expressing intraperitoneal PDX ovarian cancer models, as measured by bioluminescent imaging.

Results

Several human ovarian cell lines are susceptible to SRF231-mediated phagocytosis

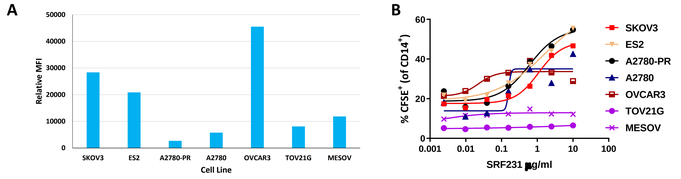

Figure 1: (A) hCD47 protein levels profiled across a panel of human ovarian cell lines via flow cytometry (FACS) using the anti-hCD47 antibody (B6H12) and mIgG1 Isotype Control. Relative Mean Fluorescence Intensity (MFI) reported as hCD47 – Isotype Control MFI. **(B)** CFSE-labeled human ovarian cell lines were cultured with human monocyte-derived macrophages at a 2:1 target:effector ratio in the presence of a dose response of SRF231 or hIgG4 isotype control antibody (not shown). Cocultures were incubated at 37^{o}C for 2 h. Phagocytosis was assessed via FACS as measured by the percentage of CFSE^{+} target cells within CD14^{+} macrophages.

SRF231 demonstrates single agent activity in the SKOV3 orthotopic xenograft model

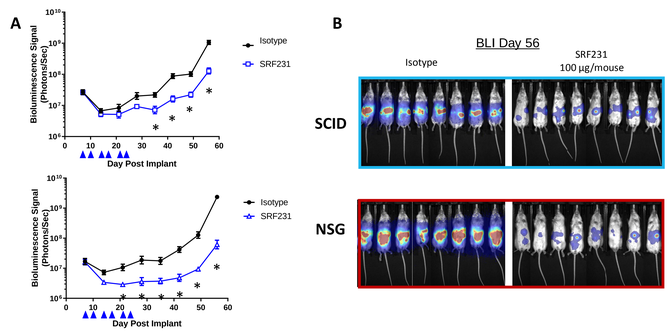

Figure 2: (A) Six to 7-week-old SCID or NSG mice were implanted intraperitoneally (IP) with 5x10^{6} SKOV3-Luc cells, sorted into groups based on tumor burden 7 days post tumor injection and dosed IP with either human IgG4 (Isotype Control) or 100 μg/mouse SRF231 (BIW x 3). Tumor growth was monitored by bioluminescence imaging (BLI) using an IVIS Spectrum and was performed on Days 7, 14, 21, 28, 35, 42, 49, and 56 post-implant. *Denotes a significant difference between SRF231 vs. Isotype control groups (unpaired t-test, p<0.05). **(B)** BLI Images depicted on Day 56 post-implant (study endpoint).

SRF231 cooperativity with oxaliplatin or doxorubicin leads to enhanced tumor cell death

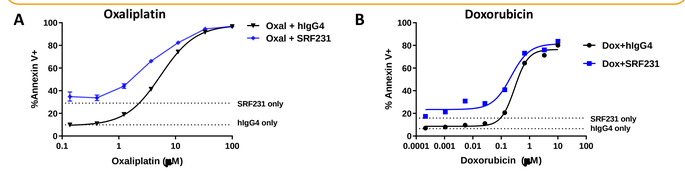

Figure 3: Jurkat cells were cultured overnight at 37^{o}C in the presence of 1 μg/mL protein-G immobilized hIgG4 or SRF231 + varying doses of **(A)** Oxaliplatin (Oxal) or **(B)** Doxorubicin (Dox). Annexin V induction on Jurkat cells was then evaluated via flow cytometry.

SRF231 with doxorubicin leads to enhanced anti-tumor activity in the OVCAR3 model

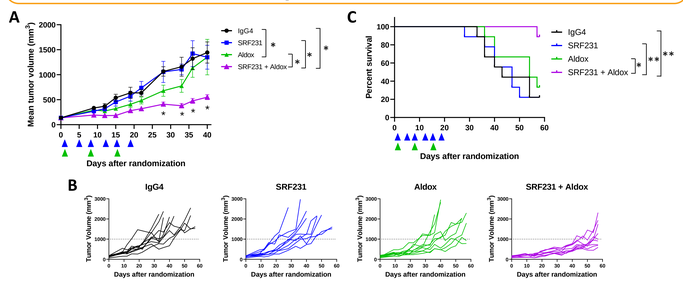

Figure 4: Six-week-old female SCID mice were implanted subcutaneously (SC) with 5x10^{6} OVCAR3 tumor cells in 50% Matrigel. When tumors reached an average volume of 100 mm^{3} mice were sorted into groups (n=9/group). Mice were dosed IP with SRF231 or human IgG4 isotype control BIWx3 (800 μg/injection). Some mice were also dosed IV with 10 mg/kg aldoxorubicin once per week for 3 weeks. **(A)** Average tumor volumes. Statistics were calculated using unpaired t-test. **(B)** Individual tumor volumes. **(C)** Survival curves compared by Log rank Mantel Cox test (*p<0.05; **p<0.01).

High CD47 expression in ovarian PDX models

Models	CD47 (% positivity)	Histologic subtype	# prior lines of chemo	Germline BRCA status	CNV Pearson Rank	BROCA mutations	PIK3CA Status
DF86	64.9	HGSOC	6	BRCA1 del exons 21-24	12	TP53, BRCA1, APC	amplification
DF83	79.8	HGSOC	4	Unknown	6	TP53, CDKN2A (copy loss)	no gain
DF216	84	adenocarcinoma	2	Wild type	11	TP53	gain
DF68	86.6	HGSOC	5	BRCA1 Q563X	9	TP53, BRCA1, PTEN	Gain
DF118	88.7	HGSOC	1	Unknown	1	TP53	no gain
DF20	89	HGSOC	0	Unknown	7	TP53, PTEN, PPM1D	amplification
DF181	92	HGSOC	7	Wild type	4	TP53, BRIP1	gain
DF101	90.7	HGSOC	2*	BRCA1 187delAG	10	TP53, BRCA1, NBN, PTEN (copy loss)	no gain

Table 1: Formalin-fixed, paraffin-embedded (FFPE) ovarian PDX tumor models were sectioned and analyzed by immunohistochemistry (IHC) to assess CD47 protein expression levels. PDX tumor samples were stained using anti-human CD47 mAb (SP279; Spring Bioscience) and slides were digitally scanned and analyzed by an image analysis algorithm (Aperio percent positive count v9.1) to quantify the degree of CD47 tumor cell expression in each PDX model (second column). Ancillary histologic, genetic, and clinical annotations for each PDX model is also provided (Liu et al. Clin Can Res 2016). HGSOC = high grade serous ovarian cancer

Results

Enhanced anti-tumor activity of SRF231 with carboplatin in PDX models

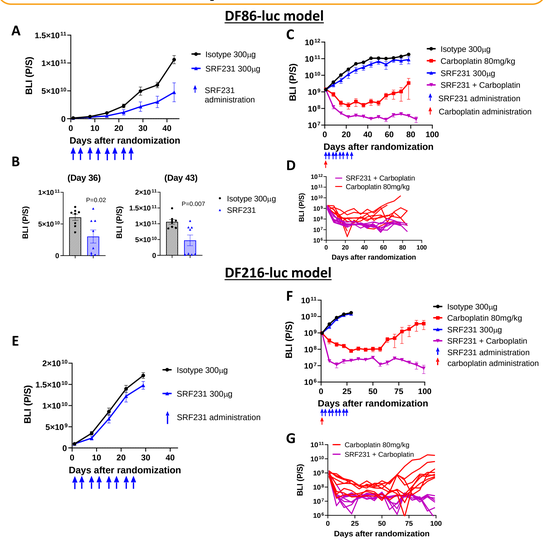

Figure 5: Female NSG mice were implanted IP with either DF86-luc or DF216-luc ovarian PDX cells. Animals were randomized into treatment groups with n=8/group. Mice were dosed IP with SRF231 or isotype control BIWx4 (300 μg/injection) or a single IP dose of carboplatin (80 mg/kg). **(A)** Single agent activity of SRF231 compared with isotype control in DF86 model. Tumor growth assessed by BLI. **(B)** Average BLI on days 36 and 43. Statistics were calculated using an unpaired t-test. **(C)** Tumor growth monitored over time by BLI. **(D)** Individual tumor volumes for single agent carboplatin and combination group. **(E)** SFR231 single agent is comparable to isotype control in the DF216 model. **(F)** Tumor growth monitored over time by BLI. **(G)** Individual tumor volumes for single agent carboplatin and combination group.

Conclusions

- Anti-CD47 directed therapy with SRF231, a fully human antibody, demonstrated the ability to significantly increase the anti-tumor activity of standard chemotherapies in xenograft and platinum-resistant PDX models of ovarian cancer.
- Further exploration of combining anti-CD47 and platinum regimens in ovarian cancer is warranted.
- Please refer to poster #2196 for more on SRF231 combinations.