1028 EX VIVO CHEMOTHERAPY RESISTANCE OF METABOLICALLY REPROGRAMMED AUTOLOGOUS TH1/TC1 CELLS (RAPA-201): TOWARDS THE CLINICAL TRANSLATION OF IMMUNE-SPARING HOST CONDITIONING

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Background Manufactured RAPA-201 cell therapy products are enriched for central-memory, polarized to Th1/Tc1, and rendered immune checkpoint-deficient and homeostatic cytokine-responsive. RAPA-201 safely mediates multiple myeloma remissions when administered after lymphodepleting conditioning and induces remissions in solid tumor patients in the post-PD-(L)-1 setting using immune-sparing conditioning [carboplatin (CBCCA) plus paclitaxel (PTX)] (separate abstract submitted by Gutierrez et al, SITC 2023; clinicaltrials.gov, NCT05144698). This latter clinical translation was motivated in part by our finding, described herein, that RAPA-201 demonstrate resistance to CBCCA plus PTX.

Methods RAPA-201 were manufactured using one-week culture in temsirolimus- and IFN-α-containing media and tested for sensitivity to chemotherapy inhibition (temsirolimus, TEM; etoposide, VP-16; paclitaxel, PTX; carboplatin, CBCCA; and topotecan, TOPO; see table 1/figure 1: concentrations and exposure intervals). Peripheral blood mononuclear cells (PBMC) served as negative control whereas positive controls consisted of pancreatic cancer cells (MIA-PaCa-2 line) and lung cancer cell lines (NCI-H1975 and NCI-H820). Multidrug resistance (MDR) activity factor (MAF) values were determined by flow cytometry efflux assays (Abcam, ab294534).

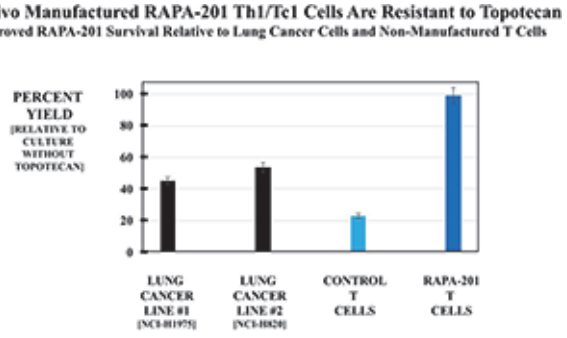
Results Relative to MIA-PaCa-2, RAPA-201 had increased resistance to TEM (table 1, EXPT #1: 9.8% vs. 69.0% yield, respectively), VP-16 (16.3% vs. 82.8% yield), and PTX (0.0% vs. 55.2% yield) and similar resistance to CBCCA (205.6% vs. 103.4% yield). Similar results were obtained in a second experiment (table 1, EXPT #2). Relative to PBMC, RAPA-201 had increased resistance to combination CBCCA + PTX (table 1, EXPT #1: 29.7% vs. 61.7% yield; EXPT #2: 63.4% vs. 138.2% yield). Relative to cellular controls (NCI-H1975, NCI-H820, PBMC), RAPA-201 had increased topotecan resistance (figure 1). MDR activity was not detected in control PBMC (MAF score, 0); in contrast, in two separate experiments, RAPA-201 had functional MDR activity for MDR1 (median MAF: 5.2, 15.9), MRP1/2 (median MAF: 6.1, 24.7), and BCRP (median MAF: 8.7, 8.2).

Conclusions RAPA-201 are globally resistant to numerous chemotherapy agents, including carboplatin, paclitaxel, temsirolimus, etoposide, and topotecan. The mechanism of RAPA-201 resistance may involve MDR activity; however, as we have previously demonstrated, modulation of intrinsic apoptotic signals is also likely operational. It is important to harness RAPA-201 chemotherapy resistance for clinical translation, as we have now successfully achieved through use of CBCCA/PTX immune-sparing conditioning to safely facilitate RAPA-201 regression of post-PD-(L)1 solid tumors. Finally, given RAPA-201 resistance to topotecan, which is in the topoisomerase I drug class commonly utilized as payload for antibody-drug-conjugates (ADC), it will be important to clinically translate combination therapy involving RAPA-201 and ADC agents.

Abstract 1028 Table 1 Ex Vivo Manufactured Rapamycin-Resistant Thl/Tcl Cells (RAPA-201): Chemotherapy Resistance

Comparison to Resistant Human Tumor Cells	Cell Type[1]	No Inhibitor (yield, %)[2]	Specific Chemotherapy Agent Tested[3] (% Yield Relative to No Inhibitor) TEM	VP-16	PTX	CBCCA
EXPT #1	MIA-PaCa-2	100	9.8	16.3*	0.0	205.9
	RAPA-201	100	69.0	82.8	55.2	103.4
EXPT #2	MIA-PaCa-2	100	N/A	6.4**	1.9	98.5
	RAPA-201	100	N/A	38.6	22.7	32.0
Comparison to Human T Cells (Unmanipulated PBMC)	**Cell Type[4]**	**No Inhibitor (yield, %)**	**Combination CBCCA + PTX[5] (% Yield Relative to No Inhibitor)**			
EXPT #1	PBMC	100	29.7			
	RAPA-201	100	61.7			
EXPT #2	PBMC	100	63.4			
	RAPA-201	100	138.2			

[1]MIA-PaCa-2, human pancreatic cancer cell line ; RAPA-201, primary human $CD4^+/CD8^+$ T cells manufactured by one-week culture containing temsirolimus (2μM) and IFN-α (20,000 IU/mL).
[2]MIA-PaCa-2 or RAPA-201 cells were plated for 48-hrs in the absence of chemotherapy; the resultant cell yield was operationally-defined as 100 (100%).
[3]MIA-PaCa-2 or RAPA-201 cells were plated for 48-hrs in the presence of the following chemotherapy agents, with the results expressed as cell yield relative to the no inhibitor control culture: TEM, temsirolimus (2μM); VP-16, etoposide (0.5 μM* or 50 μM**); PTX, paclitaxel (5 nM); CBCCA, carboplatin (2 μg/mL) .
[4]PBMC, human peripheral blood mononuclear cells.
[5]CBCCA + PTX, 48-hr culture in carboplatin (2 μg/mL) plus paclitaxel (5 nM).



Abstract 1028 Figure 1 The NCI-H1975 and NCI-H820 lung cancer cell lines were maintained in exponential growth phase and then subjected to further culture in complete media with or without addition of topotecan (30 nM). At 72-hours of culture with or without topotecan, absolute numbers of viable lung cancer cells were enumerated using flow cytometry and nucleocounter instruments, with results expressed as the percent of viable cells relative to the media control condition. For the T cell cultures, non-manufactured $CD4^+$ and $CD8^+$ T cells ('Control') or ex vivo manufactured RAPA-201 T cells from a patient with non-small cell lung cancer were brought into growth phase using anti-CD3/anti-CD28 co-stimulation combined with cytokine support (IL-2, IL-7, IL-15). After 48-hours, control or RAPA-201 T cells were then subjected to further culture in cytokine-containing media with or without addition of topotecan (30 nM). At 72-hours of culture with or without topotecan, absolute numbers of viable T cells were enumerated using flow cytometry and nucleocounter instruments, with results expressed as the percent of viable cells relative to the media control condition.

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