6254: The discovery and preclinical characterization of the SAM-competitive PRMT5 inhibitor UCT-000445

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BACKGROUND

- PRMT5 is a methyltransferase that symmetrically dimethylates arginine residues using a cofactor called SAM
- PRMT5 dimethylates several histone proteins and regulates the expression of certain genes; dysregulation of PRMT5 causes epigenetic changes that are implicated in many cancers
- Inhibition of PRMT5 can reverse these epigenetic changes and has anti-proliferative effects in many human cancer models
- Some cancers harboring an *MTAP*-deletion genotype have higher levels of the cell metabolite MTA, and MTA can partially occupy the SAM-binding site of PRMT5
- Inhibition of the methyltransferase activity of PRMT5 has been achieved with three modalities: (1) small molecule occupation of the arginine-binding site ("substrate-competitive"); (2) small molecule occupation of the SAM-binding site ("SAM-competitive"); and (3) small molecules that cooperatively occupy the SAM-binding site with MTA ("MTA-cooperative")
- We evaluated the therapeutic potential of each modality by screening benchmark inhibitors against 500+ human cancer cell lines of various histological origins
- **We found that the SAM competitive modality had the greatest therapeutic potential because of its more potent anti-proliferative effect against a broader range of cell lines**
- **We developed a novel, potent and more selective SAM-competitive PRMT5 inhibitor (UCT-000445) using our proprietary chemistry platform and structure-based design**
- JNJ-64619178, a SAM-competitive PRMT5 small molecule inhibitor that is currently in Phase 1 clinical evaluation, was used as a benchmark

MTAP-NULL RESPONSES TO MOLECULARLY TARGETED AGENTS

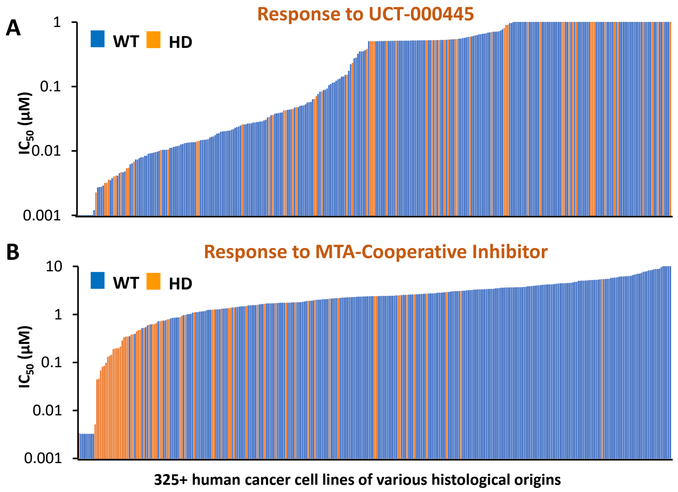

Figure 1. 325+ human cancer cell lines of various histological origins were treated continuously during a 7-day growth assay. Anti-proliferative effects are reported as IC_{50}s (μM). "WT" are *MTAP*$^{+/+}$ cell lines and "HD" are *MTAP*$^{-/-}$ cell lines. **(A)** Results from cell lines treated with UCT-000445. Similar results were obtained with a chemically distinct SAM-competitive PRMT5 inhibitor (data not shown). **(B)** Results from cell lines treated with an MTA-cooperative PRMT5 inhibitor. Similar results were obtained with a chemically distinct SAM-competitive PRMT5 inhibitor (data not shown).

- Knockdown or knockout of *PRMT5* in an *MTAP*$^{-/-}$ background leads to cell death and/or a major loss of cell proliferation[1,2]
- This observation suggests that the *MTAP*$^{-/-}$ genotype could be used as a marker of sensitivity to PRMT5 inhibition
- **Paradoxically, this phenotype was not reproduced with a substrate-competitive inhibitor[1], nor could we reproduce it with several SAM-competitive inhibitors or MAT2A inhibitors** (data not shown)
- *MTAP*$^{-/-}$ genotype is predictive of sensitivity to MTA-cooperative inhibitors[3,4,5]
- **We profiled MTA-cooperative inhibitors and found that only about 30% of 75 *MTAP*$^{-/-}$ human cancer cell lines had an IC_{50} of less than 0.5 μM, and anti-proliferative effect was seen in a surprisingly large number of *MTAP*-normal cell lines**

INTERMITTENT DOSING ALEVIATES HEMOLYTIC ANEMIA WHILE MAINTAING ANTI-TUMOR EFFECT

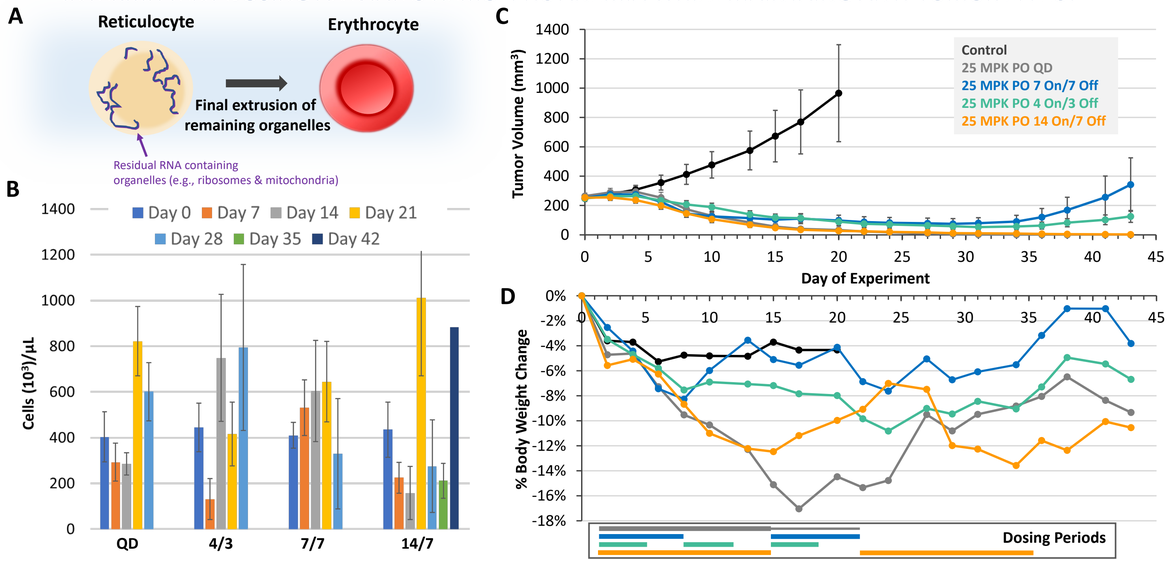

Figure 4. (A) Reticulocytes are the immediate precursor to erythrocytes and a surrogate for early hemolytic anemia. **(B – D)** CD-1 athymic nude mice subcutaneously implanted with FaDu. **(B)** Reticulocyte levels were quantified in triplicate from animals treated with UCT-000445 either QD, 4 days on/3 days off, 7 days on/7 days off or 14 days on/7days off. Blood samples were taken from animals at the indicated timepoints and CBCs were performed for each sample. Note the rapid rebound in reticulocyte levels upon inhibitor holiday. **(C)** Tumor response data. **(D)** Animal weight change data. The box below the line plot indicates the dosing periods of each arm.

SPECIFICITY, POTENCY & OTHER PROPERTIES OF UCT-000445

INHIBITION (%) OF METHYLTRANSFERASE

	PRMT5 IC_{50}	PRMT 1	PRMT 3	PRMT 4	PRMT 5	PRMT 6	PRMT 7	PRMT 8	PRMT 9	G9a
UCT-000445	25 nM	6	0	13	100	2	3	8	0	14
JNJ-64619178	32 nM	14	13	3	99	6	4	0	77	31

Table 1. IC_{50} against PRMT5 methyltransferase activity was measured in a cell-free enzymatic activity assay. Enzymatic inhibition of other MTs was assessed with similar cell-free assays at various concentrations of each inhibitor (inhibition at 200 nM presented) and are reported as % inhibition.

	k_a (1/Ms) "k_{on}"	k_d (1/s) "k_{off}"	K_D (M)
JNJ-64619178	2.06E+06	1.66E-04	8.09E-11
UCT-000445	1.99E+06	9.85E-05	4.96E-11

Table 2. Characterization of the association between inhibitors and a recombinant, chemically biotinylated PRMT5:MEP50 complex using SPR. UCT-000445 has enhanced retention time ("k_{off}") as compared to the benchmark. R_{max} and Chi^2 were within acceptable ranges.

	Avg. Cell IC_{50} (nM)	Hepatocyte $T_{1/2}$ (mins)	P_{app} (10^{-6} cm/s) A to B
UCT-000445	2.6	70 (Rat) 113 (Mouse)	0.360
JNJ-64619178	3.1	31 (Rat) 38 (Mouse)	0.805

Table 3. Evaluation of *in vitro* and cellular properties. The "Avg. Cell IC_{50}" values were calculated from 50 cell lines of various histological origins evaluated in a 7-day growth assay. P_{app} data were obtained using a Caco-2 cell permeability assay (WuXi AppTec, Wuhan, China).

- PRMT5, 7 & 9 are Type II PRMTs; the role of PRMT7 & 9 in cancer cell proliferation is not well established, and therefore, targeting them may not be necessary
- Our inhibitor spares PRMT9 while potently inhibiting PRMT5 **(Table 1)**; additionally, enzymatic inhibition of 42 recombinant human histone or methyltransferase complexes was evaluated, and none showed greater than 25% inhibition at 200 nM UCT-000445 (data not shown)
- **The greater specificity of UCT-000445 may give it a wider therapeutic index then that seen with JNJ-64619178**
- **Notably, UCT-000445 has the same anti-proliferative effect as the benchmark (Table 3), suggesting that PRMT9 and G9a inhibition are dispensable for therapeutic action**

UCT-000445 REDUCES DIMETHYLATION OF PRMT5 SUBSTRATES

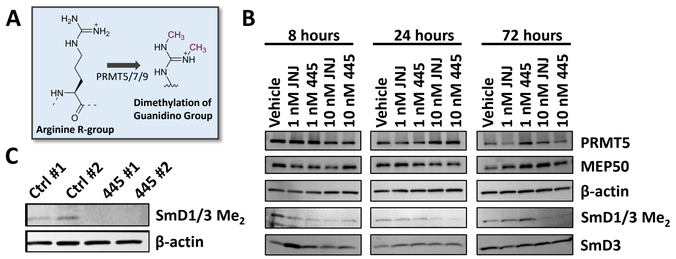

Figure 2. Dimethylation of SmD1/D3 proteins ("SmD1/D3 Me_2") was assessed by western blot as a pharmacodynamic marker of PRMT5 inhibition, which was previously validated[6,7]. SmD3 and β-actin are loading controls. **(A)** Schematic of methyl transfer to target arginine by Type II PRMTs. Note that Type I PRMTs can mono-methylate target arginine before a Type II PRMT transfers the second methyl. **(B)** FaDu cells were treated for the indicated time and proteins of interest were measured by western blot. "JNJ" = JNJ-64619178 and "445" = UCT-000445. **(C)** CD-1 athymic nude mice bearing UPCISCC125 xenografts were treated QD with vehicle control (Ctrl) or 12.5 mg/kg UCT-000445 (445) for 21 days and tumor lysates were collected. Lysates from two animals of the Ctrl group and two animals of the 445 group are presented.

UCT-000445 PRODUCES ANTI-TUMOR EFFECTS IN VARIOUS SOLID TUMOR MODELS

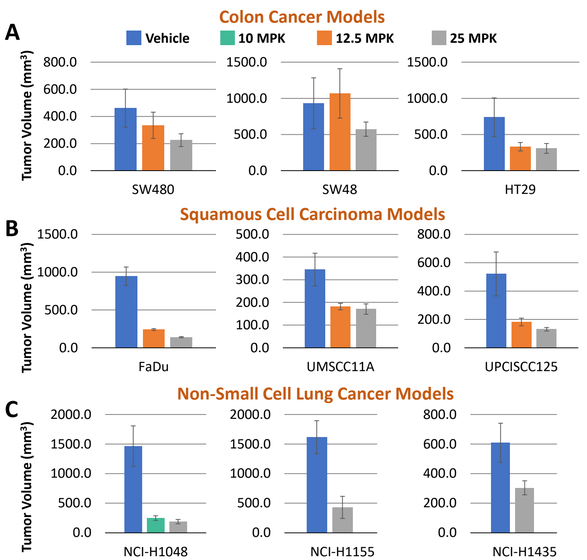

Figure 3. CD-1 athymic nude mice subcutaneously implanted with **(A)** human colon cancer cell line models, **(B)** human squamous cell carcinoma models or **(C)** human lung cancer cell line models were treated with vehicle or with the indicated dose of UCT-000445 PO QD. All studies were performed with 8 animals per arm. Tumor volumes were determined using caliper measurements and recorded in mm^3.

SUMMARY & CONCLUSIONS

- UCT-000445 is a highly selective and potent SAM-competitive PRMT5 inhibitor
- *MTAP*-deletion is not predictive of sensitivity to SAM-competitive PRMT5 inhibitors
- Even at sub-MTD doses, UCT-000445 has a significant anti-tumor effect against multiple solid tumor types
- The anti-proliferative effects of UCT-000445 in xenograft models are sustained for approximately 2 weeks after drug withdrawal
- This sustained effect allows intermittent dosing regimens to have efficacy equivalent to that of continuous dosing
- Moreover, drug holiday allows erythrocyte precursor cells to rebound, reversing the hemolytic anemia observed in animal models
- This study suggests that SAM-competitive PRMT5 inhibitors have the potential to provide benefit to patients with certain solid tumor types regardless of their tumors' *MTAP* gene status
- Our preclinical models support the use of intermittent dosing strategies to ameliorate potential side effects like cytopenias
- 1200 Pharma is preforming IND-enabling studies and plans to open a Phase 1 clinical trial in early 2024

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