SAT-122, a potential first-in-class, potent, small-molecule disruptor of RAD51-BRCA2, attenuates RAD51 foci formation and tumor progression in preclinical models

FPN : 35P

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Background

The DNA damage repair pathway plays a crucial role in signalling for effective DNA repair and cell cycle progression. DNA double-strand breaks (DSBs) are primarily repaired by homologous recombination. Acting downstream of ATR, ATM and PARP, RAD51 is a central recombinase in HR-mediated DDR pathway that participates in DSB repair *via* interaction with BRCA2, followed by its nuclear translocation. RAD51:BRCA2 interaction disruptors are first-in-class anticancer agents with therapeutic potential in refractory solid tumors. The sensitivity to RAD51:BRCA2 disruption is high in cells with high Replication Stress, such as **oncogene driven tumours**, thereby making it selective for cancer cells while sparing healthy cells. Since the mechanism of action is distinct from PARP inhibition, SAT-122 continues to be **active in a PARPi resistant** setting. SAT-122 has been identified as a candidate for IND enabling studies.

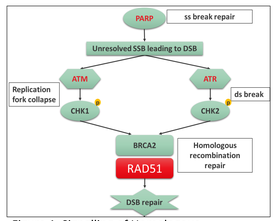


Figure 1. Signalling of Homologous recombination (HR) pathway and RAD51 is a central recombinase in HR pathway

SAT-122 binds to RAD51 and inhibits RAD51:BRCA2 interaction

Biochemical assay was developed using recombinant human RAD51 and biotin labelled BRC4 (domain of BRCA2 having highest affinity for RAD51). Compounds were tested for inhibition of RAD51:BRCA2 interaction

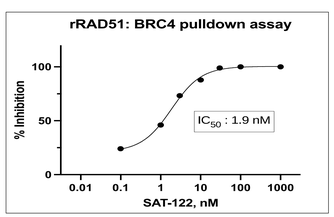


Figure 1:SAT-122 inhibits RAD51: BRC4 interaction; IC_{50} of 1.9 nM

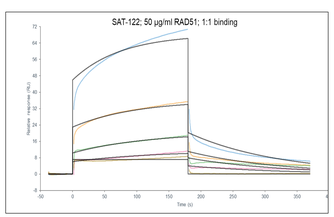


Figure 2: Binding of SAT-122 to RAD51 was measured using Surface Plasmon Resonance

Specificity within DDR pathways and Selectivity over kinases

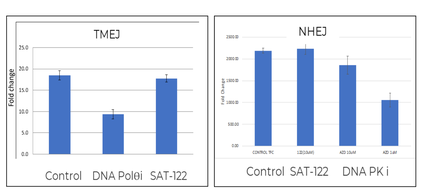


Figure 3: SAT-122 does not inhibit TMEJ and NHEJ pathways and is specific to the RAD51 pathway

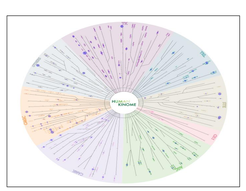


Figure 4 : SAT-122 does not have any activity at 1µM against a 345 kinase panel (Reaction biology)

SAT-122 inhibits RAD51: BRCA2 interaction in cells and modulates HR pathway genes

Disruption of RAD51:BRC4 interaction in a cellular pull down assay

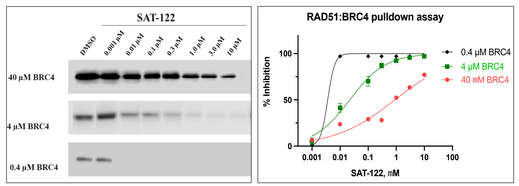


Figure 5: SAT-122 disrupts RAD51: BRCA2 interaction in MDA-MB-231 cells with an IC_{50} of 20 nM (corresponding Biochemical IC_{50} is 1.9 nM; Fig.1). The potency of SAT-122 correlates with concentration of BRC4, thereby confirming the specificity of interaction

Nanostring® expression analysis was done upon treatment with SAT-122

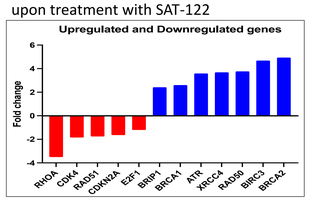


Figure 6: SAT-122 demonstrates modulation of RAD51 pathway genes

SAT-122 inhibits cell proliferation in a panel of cell lines and synergy with Olaparib

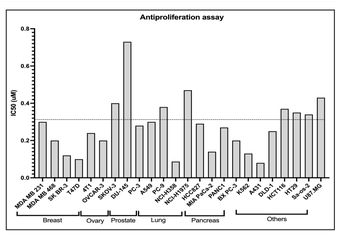
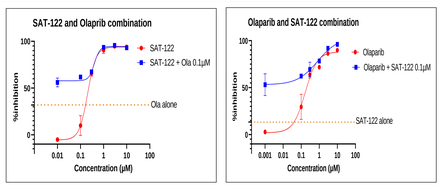


Figure 6 : SAT-122 shows potent anti proliferative activity across multiple solid tumor cell lines



BLISS synergy Score (>1 signifies Synergy)	SAT-122 DRC + Ola	Ola DRC+ SAT-122
	7.7	9.1

Figure 7 : SAT-122 demonstrates synergistic anti proliferative effect with Olaparib (PARP1 inhibitor)

SAT-122 reduces nuclear RAD51 foci and increases γH2AX

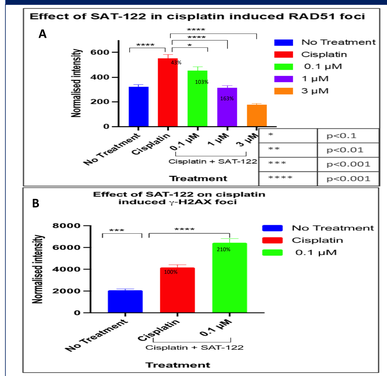


Figure 7: (A) SAT-122 demonstrates dose dependent reduction of RAD51 foci (B) SAT-122 demonstrates increase in γH2AX, indicating persistent DNA Damage

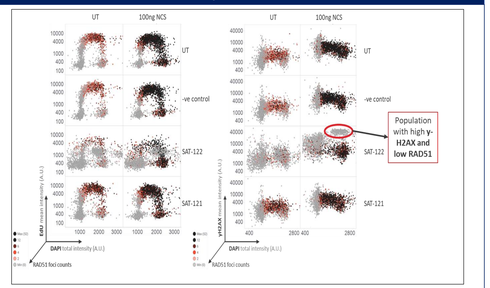


Figure 8 : Quantitative image-based cytometry (QIBC) analysis shows

- SAT-122 reduces nuclear RAD51 foci and arrests cells in late S and G2 phase; specifically, γ-H2AX high sub-population shows significant reduction in RAD51 foci
- Inactive compound SAT-121 and negative control do not have any effect on RAD51 foci and γ-H2AX

ADME and PK profile of SAT-122

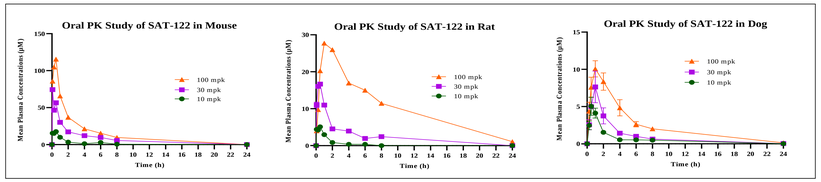


Figure 9: SAT-122 PK profile across species

SAT-122 shows desirable ADME properties (good solubility across pH, metabolically stable across species, no inhibition observed with 7 CYP isoforms) with no hERG liability. SAT-122 is dose proportional with exposures well above IC_{90} concentrations.

Efficacy and tolerability of SAT-122

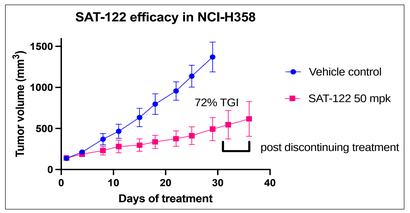


Figure 10: SAT-122 demonstrated 72% TGI in Nci-H358 xenograft model

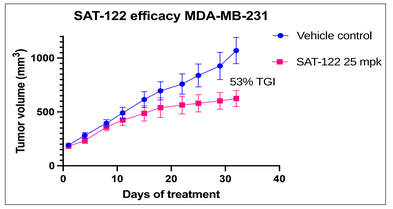


Figure 11 : SAT-122 demonstrated 53% TGI in MDA-MB-231 xenograft model

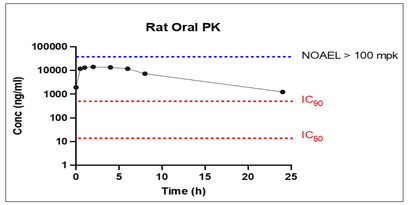


Figure 13: SAT-122 has a wide therapeutic window

- SAT-122 demonstrated efficacy in multiple xenograft models. Single agent efficacy is superior to other reported DDR pathway inhibitors.
- 14-day repeat dose toxicity study in rats showed no clinical signs or significant body weight changes and no change in clinical parameters in both the genders
- SAT-122 has a wide therapeutic window (NOAEL >100mpk and LD_{50} > 1000 mpk)

Summary

- SAT-122 is a first in class, potent inhibitor of RAD51: BRCA2 interaction with an IC_{50} of 1.9 nM
- SAT-122 demonstrates potent anti proliferative activity across panel of cell lines
- Direct PD demonstrated by a reduction of nuclear RAD51 foci; additionally, single cell analysis by QIBC shows cells with persistent DNA damage have minimal nuclear RAD51 foci.
- SAT-122 has desirable ADME properties and dose proportional PK across species
- SAT-122 inhibits only the RAD51 mediated HR pathway (and not the other DDR pathways i.e. NHEJ and TMEJ) – potentially sparing non-targeted cells
- NanoString gene expression profile upon treatment with SAT-122 shows significant modulation of RAD51 pathway genes, in line with the mechanism of action
- SAT-122 demonstrates efficacy in multiple xenograft models with no effect on body weight/clinical signs
- 14-day toxicity studies show no adverse events and no drug accumulation
- Synergy with Olaparib, and retention of activity in Olaparib resistant cells opens a clinical path in HRD cancers
- SAT-122 can be a treatment option for patients bearing RAS/EGFR mutant tumors characterized by high replication stress, both as a single agent and in combinations with SOC.

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