



Sensitivity to SNS-595 is Related to Activation of Double Strand DNA Break Repair Pathways Including Homologous Recombination

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ABSTRACT

SNS-595 is a replication-dependent agent that induces DNA damage, irreversible G2 arrest and apoptosis by intercalation of DNA and poisoning of topoisomerase II. SNS-595 is under clinical investigation in acute myeloid leukemia and ovarian cancer. Clinical responses have been observed in these indications (Lancet et al., ASH 2007; McGuire et al., SGO 2008), as well as in NSCLC and SCLC (Burriss et al., ECCO 2007). The involvement of DNA-PK in repair of SNS-595-induced DNA damage has been reported (Hyde et al., 2006). Here, the DNA repair pathways that influence cell sensitivity to SNS-595 are further characterized, revealing a role for homologous recombination repair (HRR) in the repair of double-strand breaks induced by the agent.

SNS-595 was profiled by the growth inhibition assay in the NCI60 cancer cell screening panel (Shoemaker, 2006) to identify factors influencing cellular response. The potential relationship between SNS-595 activity and DNA repair pathway integrity was assessed by correlating the GI_{50} in the NCI60 panel with the baseline RNA expression profiles of the untreated cells. These analyses suggested possible involvement of several DNA repair pathways including HRR, nucleotide excision repair (NER) and single-strand break (SSB) repair by base-excision repair (BER).

A role for HRR in sensitivity to SNS-595 was validated in an MTT assay using CHO cell lines mutant and corrected for RAD51D, Rad51 and its paralogs (including Rad51D) are proteins required for HRR. HRR-deficient cells were sensitized to SNS-595, decreasing the EC_{50} ~23-fold (0.142 μ M vs. 0.006 μ M). A similar decrease in EC_{50} for SNS-595-induced G2 arrest was noted. The repair of DNA damage was both compromised and delayed in the HRR deficient cell line, and at 1 μ M SNS-595 was found to overwhelm the repair machinery in both HRR-deficient and competent cells. In HT-29 cells Rad51 co-localized with γ H2AX in nuclear foci following SNS-595 treatment. Additional cell lines and primary cancer cells will be characterized.

Several cancer cell lines with increased GI_{50} expressed increased ERCC6, a protein required for transcription coupled NER. However, no general correlation between ERCC6 expression and sensitivity to SNS-595 was found by MTT analysis of NCI-H226, SKOV-3 and MDA-MB-231. We also evaluated the role of SSB repair in response to SNS-595. Studies of matched cell lines mutant and corrected for XRCC1, a protein required for BER of SSBs, showed no difference in sensitivity.

In conclusion, sensitivity to SNS-595 was inversely related to the ability of the cell to repair double-strand breaks by HRR. Further studies will address the relative roles of NHEJ and HRR in response to SNS-595 induced DNA damage to identify potential patient stratification biomarkers.

SNS-595-Induced DNA Damage Repair Utilizes Homologous Recombination

Figure 2: DNA double-strand breaks are repaired by HRR and NHEJ. HRR requires BRCA and RAD51

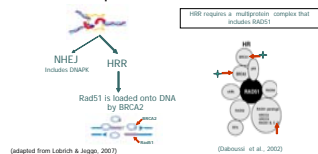
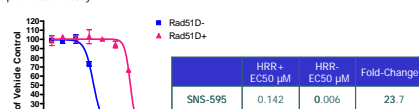


Figure 3: Cells deficient in HRR are sensitized to SNS-595. CHO cells deleted and complemented for Rad51D expression compared in proliferation assay



CHO cells mutant and complemented for RAD51D (hence mutant and competent for HRR) provide a tool to dissect the role of HRR in repair of SNS-595 induced DNA damage. (Cells kind gift of Larry Thompson, Leamora Labs, HSE, et al., 2006)

Figure 4: The HRR pathway is overwhelmed following 6 hour treatment with 1 μ M SNS-595

1 μ M SNS-595 for 6 hours is sufficient to overwhelm the DNA damage repair induced by SNS-595 in HRR competent cells. At concentrations <1 μ M, cells deficient in HRR are unable to completely repair all damage and are delayed in damage repair. DNA damage persists to 40 hours following 6 hour treatment

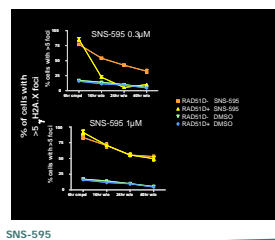


Figure 5: HRR-deficient cells show G2 arrest at lower concentrations of SNS-595 than HRR-competent cells. Parental cell line shows similar results to the reconstituted Rad51D cell line, with initial G2 arrest at 0.037 μ M (not shown).

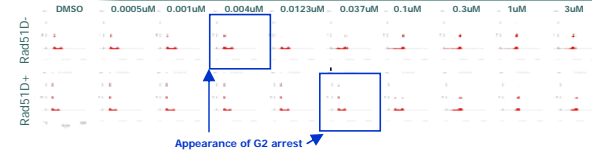
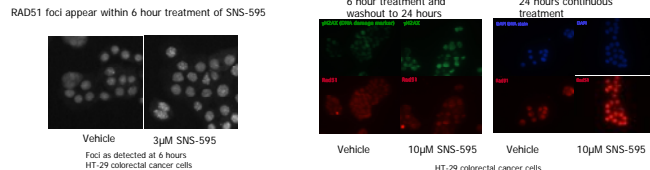
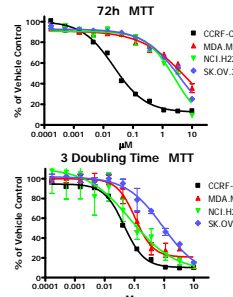


Figure 6: SNS-595 Induces the Formation of RAD51 Foci within 6 hours. These foci remain to at least 18 hours post-treatment



ERCC6 Expression Does not Influence Cell Sensitivity to SNS-595

Figure 7: SNS-595 sensitivity correlates with cell doubling time. Cell lines with high expression of ERCC6 (required for NER) and reduced sensitivity to SNS-595 were compared with the sensitive cell line CCRF-CEM, which expresses low levels of ERCC6 mRNA

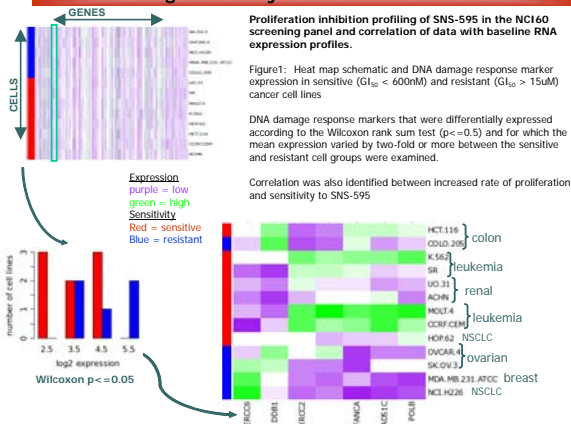


72h Proliferation (MTT) assay confirmed the NCI60 data. Allowing cells to pass through 3 doubling times equilibrated sensitivity. Cell proliferation rate, rather than variation in expression of ERCC6, influences sensitivity to SNS-595.

72h MTT	CCRF-CEM	MDA.MB.231	NCI.H226	SK.OV.3
Mean EC50 (uM)	0.050	6.00	2.46	3.70
S.D.	0.029	2.56	0.34	0.13
N	3	5	5	2

3 Doubling Time MTT	CCRF-CEM (80h)	MDA.MB.231 (5 days)	NCI.H226 (7 days)	SK.OV.3 (5 days)
Mean EC50 (uM)	0.050	0.186	0.053	0.709
S.D.	0.007	0.061	0.029	0.272
N	2	4	2	2

NCI60 Cell Panel Screen to Identify Possible Factors Conferring Sensitivity and Resistance to SNS-595



Proliferation inhibition profiling of SNS-595 in the NCI60 screening panel and correlation of data with baseline RNA expression profiles.

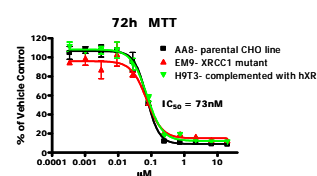
Figure 1: Heat map schematic and DNA damage response marker expression in sensitive (GI_{50} < 60nM) and resistant (GI_{50} > 15uM) cancer cell lines.

DNA damage response markers that were differentially expressed according to the Wilcoxon rank sum test (p <= 0.5) and for which the mean expression varied by two-fold or more between the sensitive and resistant cell groups were examined.

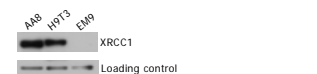
Correlation was also identified between increased rate of proliferation and sensitivity to SNS-595

Base Excision Repair (BER) Does not Influence Cell Sensitivity to SNS-595

Figure 8: XRCC1 mutant or complemented cell lines, and parental cells, show no difference in sensitivity to SNS-595



BER of single strand breaks requires the expression of the XRCC1 protein



SUMMARY & CONCLUSIONS

- SNS-595 targets replicating DNA and cytotoxicity correlates with cell proliferation rate
- The DNA damage induced by SNS-595 is repaired by HRR, and cells deficient in HRR are sensitized to the agent
 - Breast and ovarian cancers with BRCA mutations may represent highly sensitive sub-population
 - Clinically achievable exposures of SNS-595 are able to overwhelm the HRR damage response
- Broad activity has been seen in primary breast cancer biopsy samples (Poster #2830)
- Clinical responses to SNS-595 have been observed in relapsed / refractory AML and platinum-resistant ovarian cancers, as well as in lung cancers (ECCO 2007, ASH 2007, SGO 2008).
- SNS-595 is currently in a clinical phase 1b trial in relapsed / refractory AML in combination with cytarabine, and in phase 2 trials as a single agent in both platinum-resistant ovarian cancer and in elderly, untreated AML patients.