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A Novel RAD51 Inhibitor, CYT-0851, Shows Anti-Cancer Activity In Preclinical Models of AID Expressing Solid Tumors

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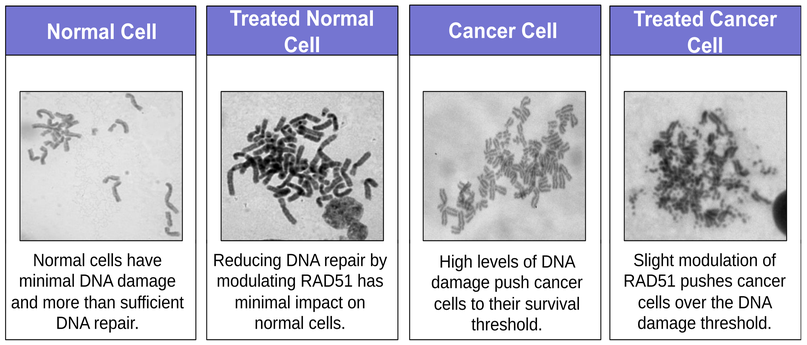
Abstract:

Activation inducted cytidine deaminase, AID (gene symbol AICDA), a DNA-directed cytidine deaminase, plays a critical role in somatic hypermutation and immunoglobulin class switching in maturing B-lymphocytes. AID is a promiscuous DNA damaging enzyme that deaminates cytidines at sites throughout the genome. AID expressing cells become critically dependent on the homologous recombination factor RAD51 to repair DNA double strand breaks that result from these off target deamination events. We have developed a novel small molecule, CYT-0851, which inhibits RAD51 response to DSBs. Here we present new preclinical characterization data of CYT-0851 in solid cancer models. We first analyzed the frequency and levels of AID overexpression in solid tumor data in The Cancer Genome Atlas. Multiple solid tumor types displayed ectopic AID expression, including breast cancer, sarcoma, melanoma, pancreatic cancer, lung cancer, and head and neck cancers. We then tested CYT-0851 in several solid tumor derived human cell lines. We observed a correlation between AID expression and activity. Cell lines with low ectopic expression of AID had EC50 values in the low micromolar range (~2μM), while those without AID expression gave EC50 values of about 5μM and higher. Next, we examined the activity of our small molecule in 14 different PDX models with various levels of AID expression. These models included renal, head and neck, lung, pancreatic, ovarian, colorectal, and breast cancer samples. We observed a wide range of tumor growth inhibition across the different models (0% to 100%+), which tended to correspond with AID expression. Taken together, these data demonstrate that CYT-0851 shows anti-cancer activity in a range of solid tumor models.

Background:

The AID-RAD51 Axis In Cancer: A Synthetic Lethality Target



Reducing RAD51 pathway activity in AID expressing cancer cells results in reduced DNA repair capacity. This results in DNA damage overload and cell death in cells experiencing high DNA damage stress. Thus, RAD51 is a synthetic lethal target in AID positive cancers.

Results:

CYT-0851 Shows Dose-Responsive Activity in Preclinical Models

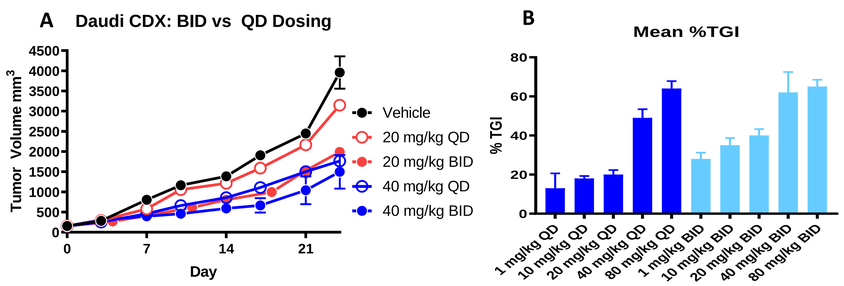


Figure 1. AID positive Burkitt's lymphoma (Daudi cell line) Cell line derived xenograft (CDX) Models. Mice were engrafted with Daudi cells which were allowed to expand to 200mm3. These mice were treated with either QD or BID Dosing (**A**). Measurements of tumor volume were taken every 3-7 days, error bars represent standard error of the mean. (**B**) At the end point %TGI was determined, the mean and SEM were plotted. CYT-0851 treatment resulted in a reduction in tumor volume ranging from about 10% at 1 mg/kg QD to +60% at 80 mg/kg QD.

Overexpression of AID across Solid Tumors is Independent of Copy Number Variation

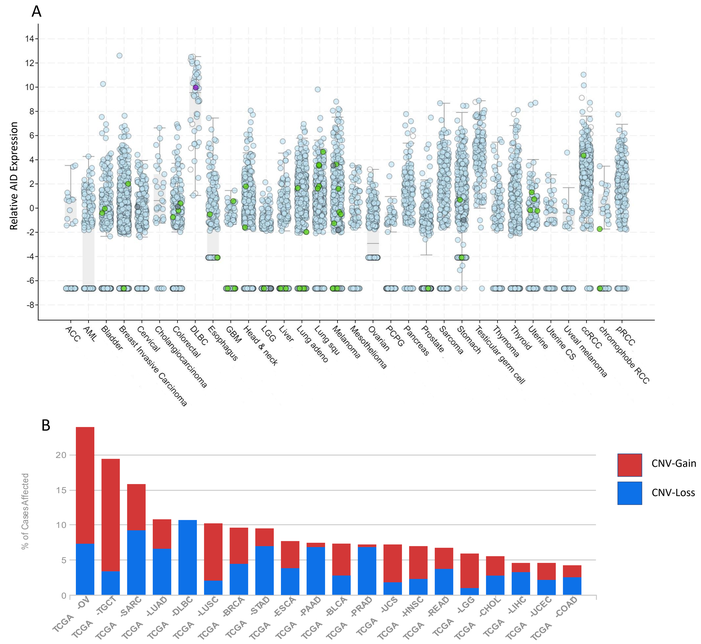


Figure 2. The Cancer Genome Atlas expression and CNV data across multiple tumor types. (A)The mRNA expression data was generated using RNA Seq V2 analysis produced by the RSEM algorithm and plotted as a log2 scale. (**B**) Copy number variation of AID was obtained from TCGA and plotted as a percentage of cases.

CYT-0851 Activity is Correlated with AID Expression

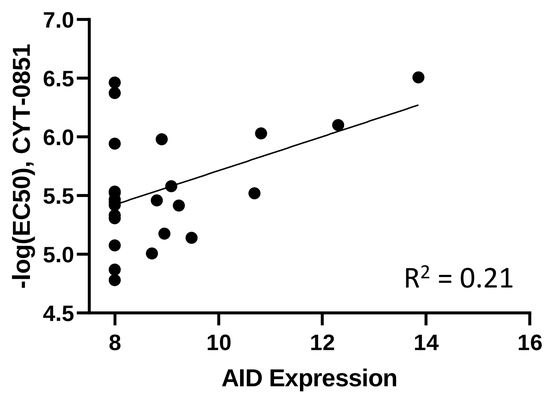
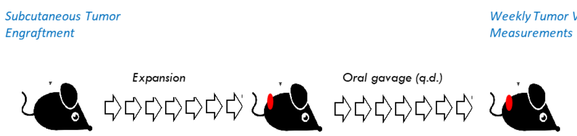
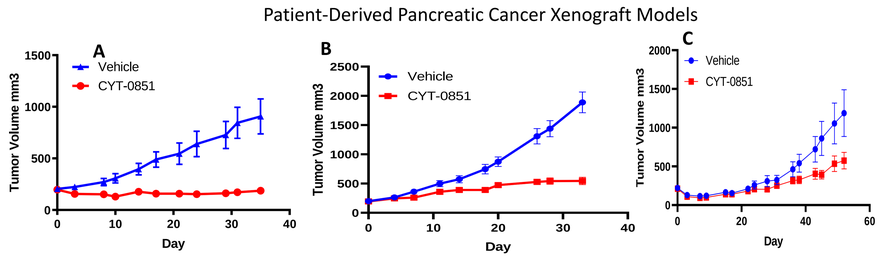


Figure 3. The expression of AID was plotted against the –log(EC50) of cells treated with CYT-0851. A linear regression was performed.

CYT-0851 Is Active In Preclinical Models Of AID Positive Tumors



Different patient derived xenografts (PDX) with varying expression levels of AID from several different tumor types were subcutaneously engrafted onto the flanks of NOD/SCID mice. The tumors were allowed to reach a volume of about 200 mm3 prior to the animals being randomized into two groups of ten animals each. One group was treated with the vehicle control while the other was treated with CYT-0851 at 80 mg/kg QD. Tumor measurements were collected bi-weekly.



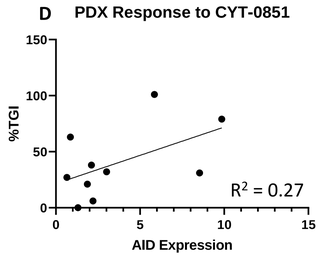


Table 1 Patient-Derived Xenograft Models

Tissue	AID Expression	% TGI
Pancreatic (A)	5.85 fold	101
Pancreatic (B)	9.85 fold	79
Pancreatic (C)	0.86 fold	63
CRC	2.11 fold	38
TNBC	3.01 fold	32
Sarcoma	8.54 fold	31
Ovarian	0.65 fold	27
NSCLC	1.87 fold	21
Esophageal	2.20 fold	6
CRC	1.31 fold	0

Figure 4. (A,B,C) NOD/SCID mice were engrafted subcutaneously with patient-derived pancreatic tumor samples and allowed to expand to 200mm^3 by caliper measurements. Animals were then dosed QD with vehicle or CYT-0851 at 80mg/kg (N=10/cohort) and tumor volumes were measured by calipers at 3-7 day intervals. Error bars represent standard error of the mean.

Conclusions:

- **Solid tumors show ectopic expression of AID, indicating that targeting the synthetic lethal interaction between RAD51 and AID may be broadly applicable across tumor types.**
- **Consistent with this, CYT-0851 shows activity in multiple solid tumor models, notably pancreatic cancer.**
- **The degree of tumor growth inhibition in vivo is partially correlated with AID expression.**
- **CYT-0851 activity in AID-low tumors indicates additional biomarkers of DNA damage which may drive sensitivity and predict response.**

References:

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- The results shown here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga

*TM, MD, and KM are employees at Cyteir Therapeutics, JV, CM, and AC are paid consultants at Cyteir Therapeutics

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