Discovery of Small Molecule Inhibitors of ADAR1

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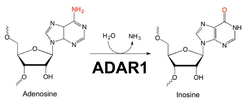
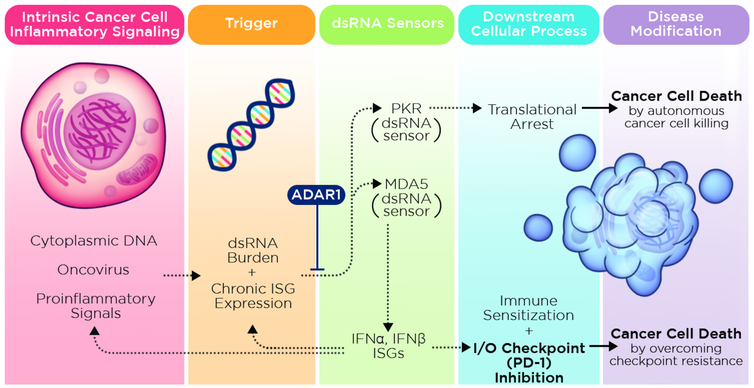
ADAR1 RNA Editor Premise: An Exciting Oncology Target in Innate Immunity

- The enzyme ADAR1 catalyzes the majority of A-to-I editing, where it has been demonstrated to effect coding sequence, miRNA function and silencing of Alu repetitive elements[1]

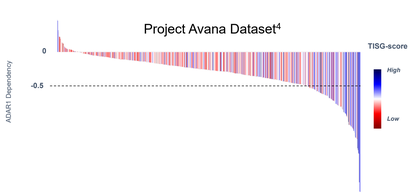


- A critical function of ADAR1 is to edit double stranded RNA (dsRNA) structures- such as Alu elements- that can activate the cytoplasmic nucleoside sensor MDA5 and induce an innate immune type I interferon (IFN) response[2,3]
- A subset of tumor cells can have higher intrinsic type I Interferon signaling and dsRNA burden due to multiple factors. Loss of ADAR1 in these cells has been shown to induce the dsRNA sensors PKR and MDA5, resulting in translational arrest and immune sensitization
- ADAR1 is thus an attractive oncology drug target for monotherapy and in combination with immuno-oncology therapy

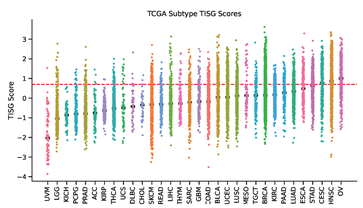


A Type I IFN Gene Signature Predicts Sensitivity to ADAR1 Knock Down

Cell Lines With Elevated Type I IFN Signaling (TISG score) are Dependent on *ADAR1*



TISG-High Score Exist Across Multiple Cancer Subtypes in the TCGA



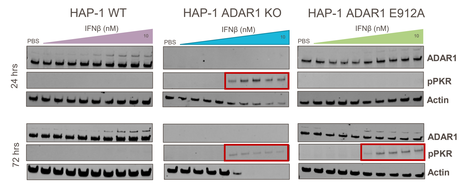
- Accent TISG score is determined by expression of a 26 gene set comprised of a subset of type I interferon –stimulated genes that predict ADAR1 dependence
- ~15-30% of primary TCGA tumors display elevated type I interferon signaling, with enrichment in HNSCC, ovarian, cervical and breast cancer

References

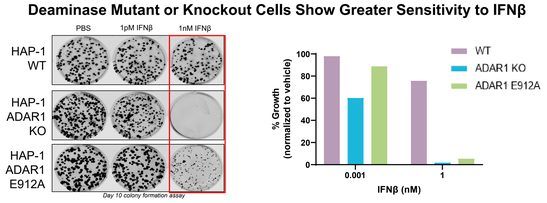
1. Eisenberg *et al*, Nature Review Genetics, 2018
2. Ahmad *et al*, Cell, 2018
3. Chung *et al*, Cell, 2018
4. Meyers *et al*, Nature Genetics, 2017
5. Park *et al*, Nature Communications, 2020

Activation of PKR and Associated Cell Death in Editing Deficient Cells Validates Small Molecule Inhibition Approach

ADAR1 Deaminase Mutation or Protein Loss Activates the dsRNA Sensor PKR



Deaminase Mutant or Knockout Cells Show Greater Sensitivity to IFNβ

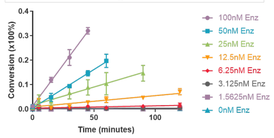


- Isogenic ADAR1 wild-type, knockout and catalytic site mutant (E912A) knock-in cell line models developed to interrogate downstream biology
- Complete loss of ADAR1 protein or loss of catalytic activity show similar activation of PKR with Interferon-β treatment
- ADAR1 loss or catalytic site mutant cells are more sensitive to exogenous Interferon-β leading to decreased growth and viability

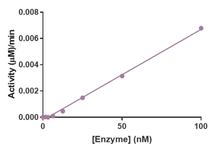
Accent ADAR1 HTS-Compatible Biochemical Assay Developed Under Balanced Conditions

- Linear progress curves over time and linear increase in reaction velocity with ADAR1 enzyme
- Robust substrate inhibition observed at higher concentrations of RNA substrate
- HTS compatible, final optimized assay conditions run at [RNA substrate] = K_M
- Tool inhibitors- including RNA product inhibitor- performed well in assay

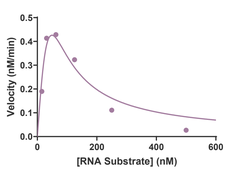
ADAR1 Enzyme Titration Reaction Progress Timecourse



ADAR1 Assay Velocity vs. Enzyme Concentration



ADAR1 Substrate K_m Determination



ADAR1 Kinetic Parameters

K_M	55.6 nM
K_i	17.2 nM
k_{cat}	0.48 hour $^{-1}$

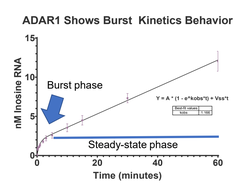
Assay performance

High-control CV	27%
Z-factor	0.66

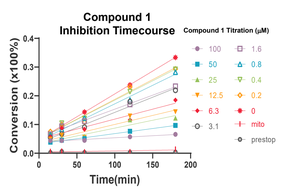
Accent ADAR1 Inhibitor Series Mechanism

Inhibition Kinetics

ADAR1 Shows Burst Kinetics Behavior

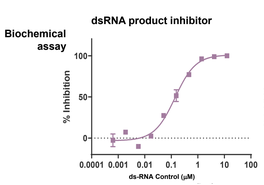


Compound 1 Inhibition Timecourse

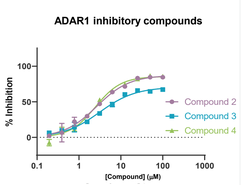


Substrate Competition Experiments

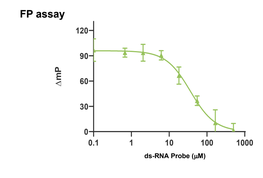
dsRNA product inhibitor

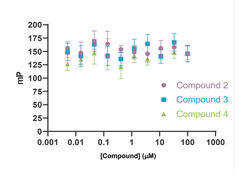


ADAR1 inhibitory compounds



FP assay

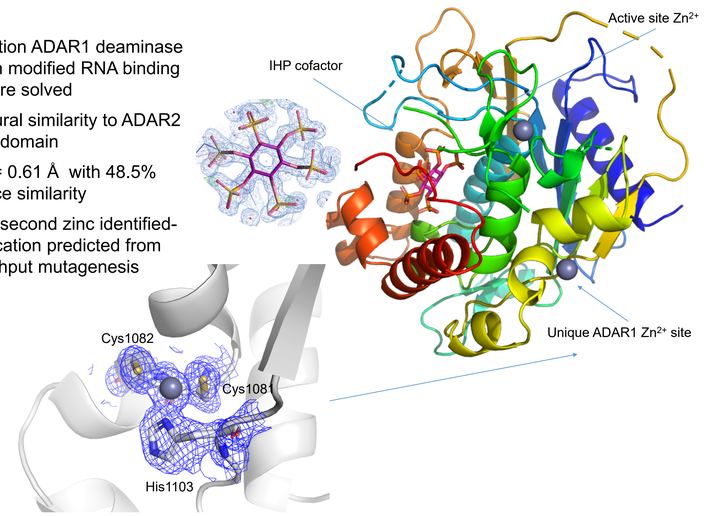




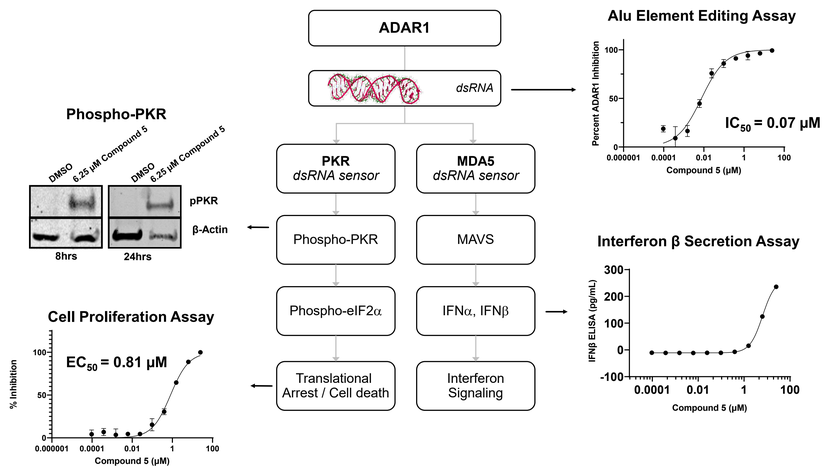
- Novel ADAR1 small molecule inhibitor series was identified by high-throughput screening
- ADAR1 displays burst kinetics behavior- compounds exclusively disrupt steady-state phase
- Series is not substrate-competitive as read out by fluorescence polarization assay

ADAR1 Structure Solved to 1.45 Å Resolution

- High resolution ADAR1 deaminase domain with modified RNA binding loop structure solved
- High structural similarity to ADAR2 deaminase domain
  - RMSD = 0.61 Å with 48.5% sequence similarity
- Location of second zinc identified- matches location predicted from high-throughput mutagenesis studies[5]



Compounds Demonstrate Inhibition of ADAR1 Editing Activity and Trigger Downstream Pathway Activation in TISG-High OE21 Cells



Conclusions

- Accent has developed a gene score (TISG) representing a subset of interferon-stimulated genes that predicts dependency on ADAR1
- Small molecule inhibitor series identified from high-throughput screening using a biochemical assay run under balanced conditions
- Series has a unique mechanism- non-competitively inhibiting the steady-state phase of the reaction
- Optimized Accent ADAR1 inhibitors show downstream effects of ADAR1 inhibition in panel of cellular assays

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