Artemis Function in DNA repair and Immunogenesis

DNA REPAIR VIDEOCONFERENCE

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The *Artemis* Gene and SCIDA

- SCIDA: Severe Combined Immunodeficiency Disease in Athabascan-speaking Native Americans
- SCIDA Incidence - I:2,000 live births (mutation frequency ~2.1%)
- Autosomal recessive nonsense founder mutation
- Deficiency in Artemis causes B-T- NK+ SCID and hypersensitivity to ionizing radiation.

Map of the Navajo Reservation

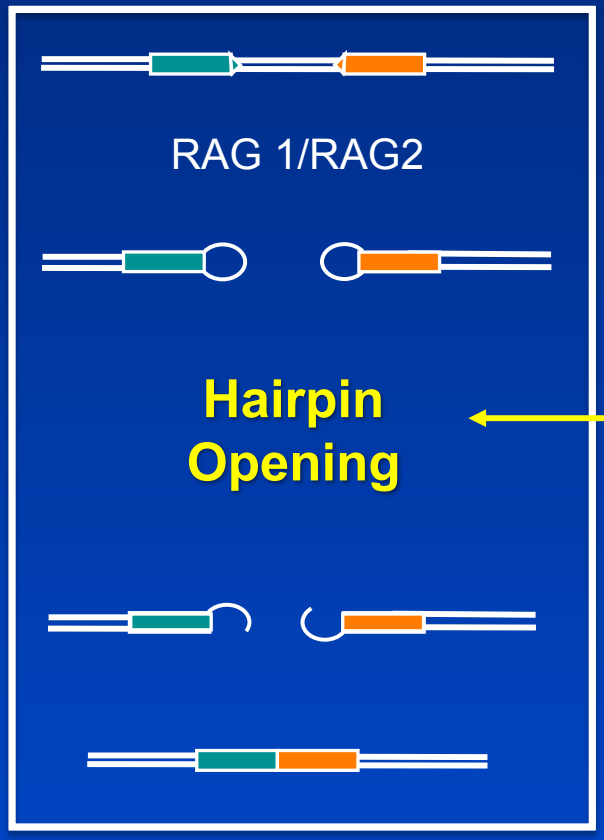
Cline Library
Special Collections and Archives Department
Northern Arizona University

A founder mutation in Artemis, an SNM1-like protein, causes SCID in Athabascan-speaking Native Americans.

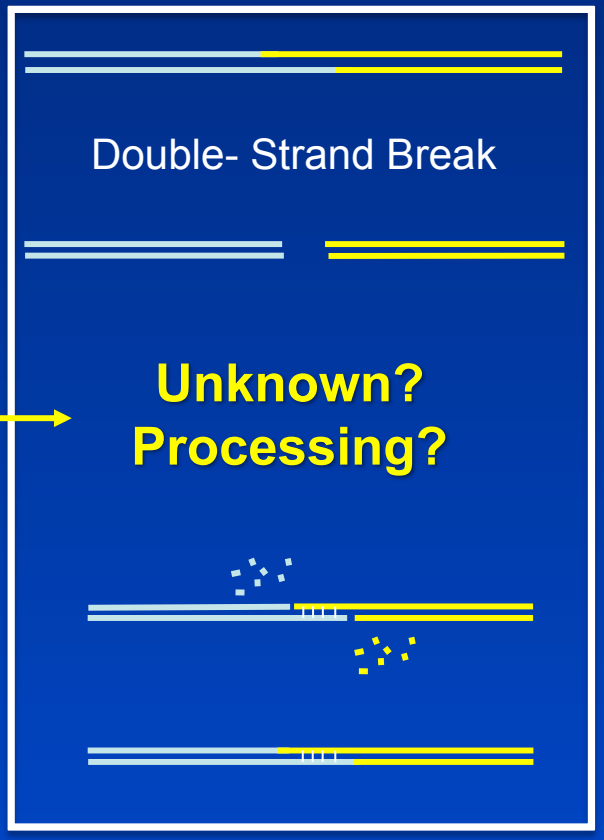
Li *et al*, J Immunol. 2002 Jun 15;168(12):6323-9

Intersect of DNA Repair and Immunity

V(D)J Recombination



Non-Homologous End Joining



Artemis
Ku70/80
DNA-PKcs

Ligase IV/XRCC4

Essential for B and T Cell Maturation

Critical for Cellular Survival

Treating Immunodeficiency in SCDA Children

The Problem

- Bone marrow transplantation is the only effective therapy for SCID.
- Both RAG and Artemis mutations present clinically as B-T- NK+ SCID.
- Effective bone marrow transplant requires immunoablative therapy (X-Rays, Chemotherapies)

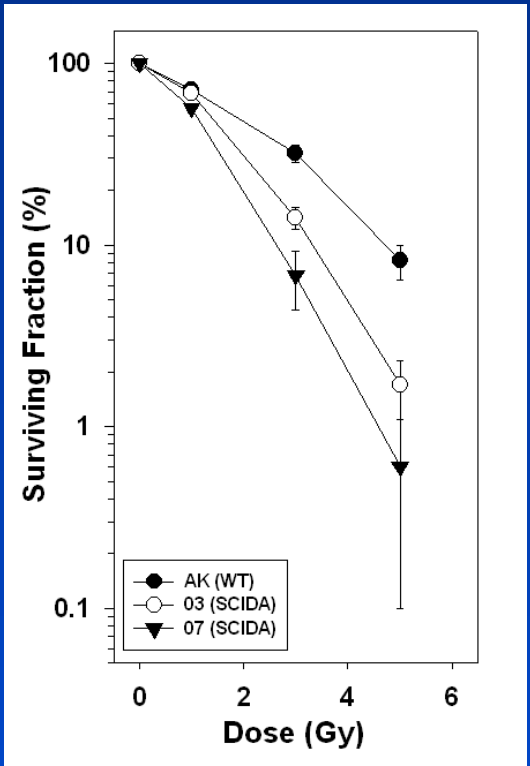
RAG SCID: Immunoablation - better BMT engraftment.

Artemis SCID: Immunoablation - poor prognosis.

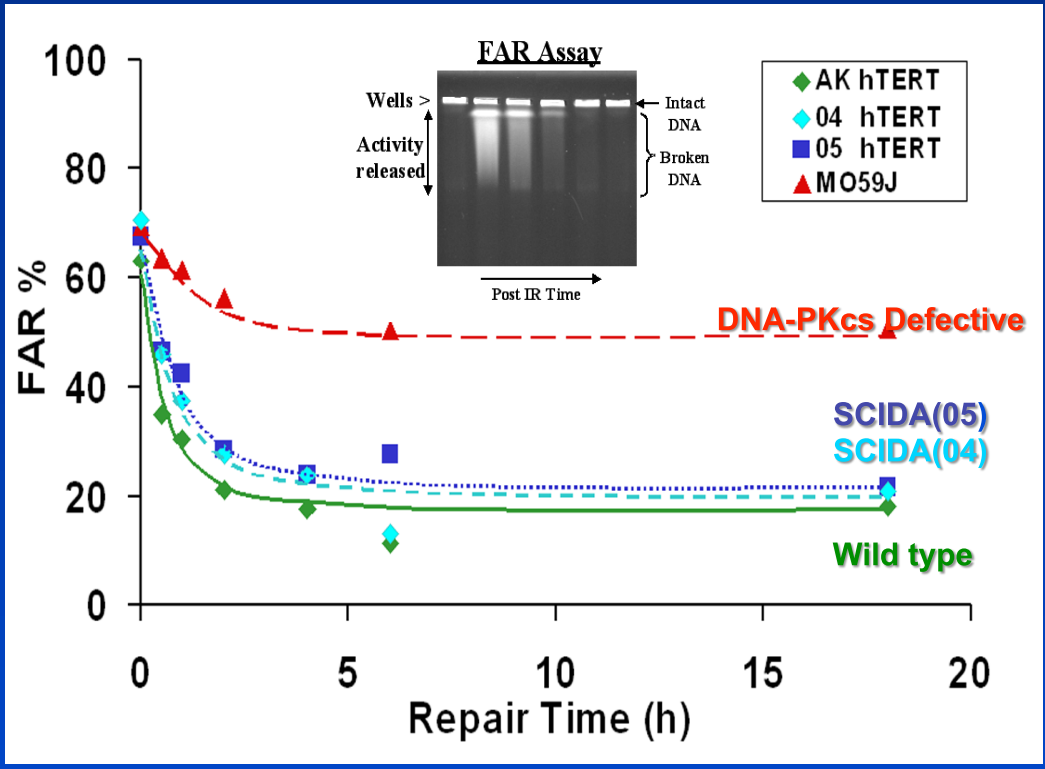
Can defining Artemis substrates allow us to identify a less toxic immunoablative therapy for Artemis-deficient SCID children?

Artemis Defects are Unlike other NHEJ Defects

Like all NHEJ defects:
SCIDA Cells are Radiation Sensitive



Unlike Other NHEJ defects:
Double-Strand Break Repair is NOT Grossly Defective in SCIDA cells

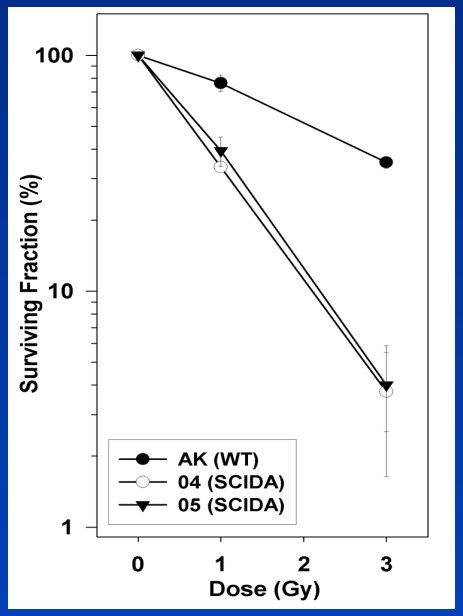


Wang *et al*, DNA Repair (Amst). 2005 May 2;4(5):556-70.

Hypothesis: **Artemis function is essential for repair of a subset of DSBs.**

SCIDA Cells are Not Equally Sensitive to IR and Etoposide Induced DSBs

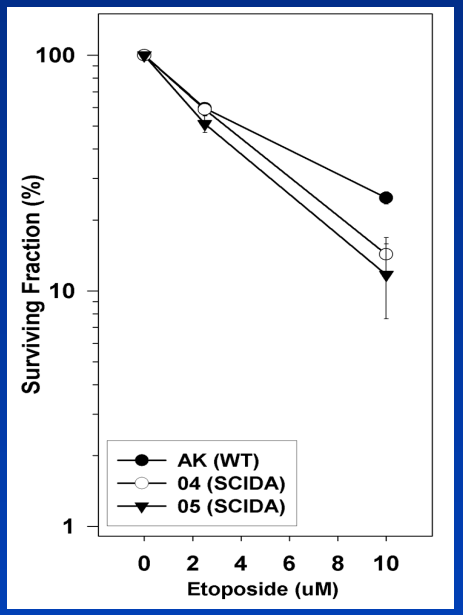
X-Rays



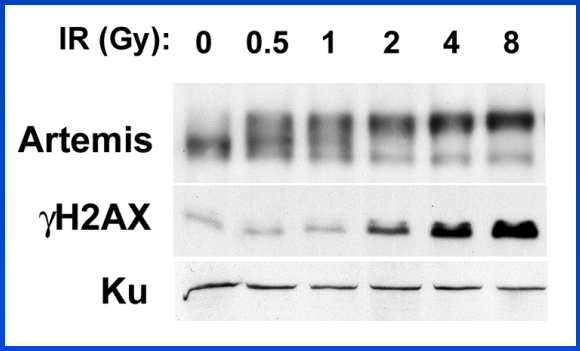
Heterogeneous DSBs
(~50% 3-PG termini)

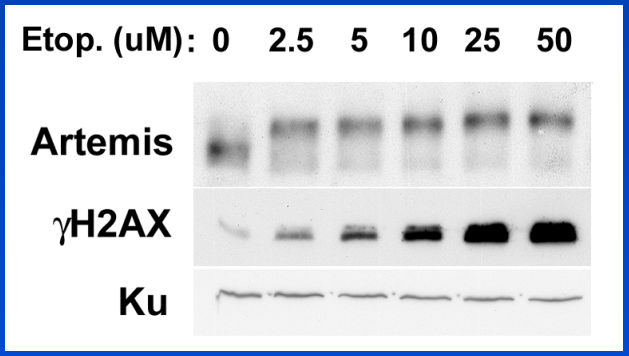
Homogeneous DSBs
5' blocked termini

Etoposide



Damage dose monitored
by phosphorylation events



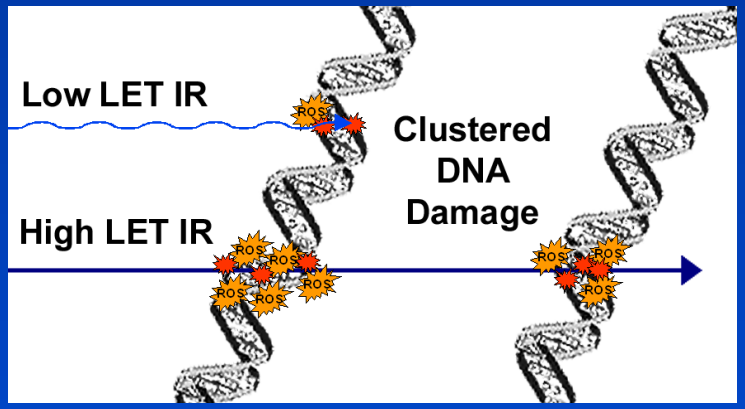


So What Are Artemis Substrates In Vivo?

1) Only a small subset of IR induced DSBs require Artemis.

2) Are more abundant after IR than etoposide treatments.

3) Are a significant determinant of cellular survival.

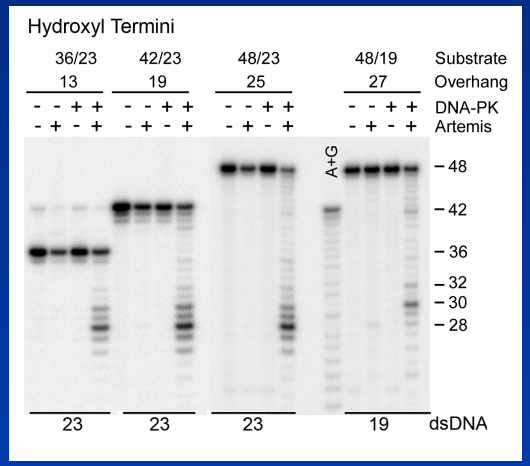


Characteristics of IR DSBs

- Heterogeneous Breaks and Termini
- Clustered Damage on DNA
- Randomly Distributed in Genome
- 3' Phosphoglycolates at Termini (~50%)

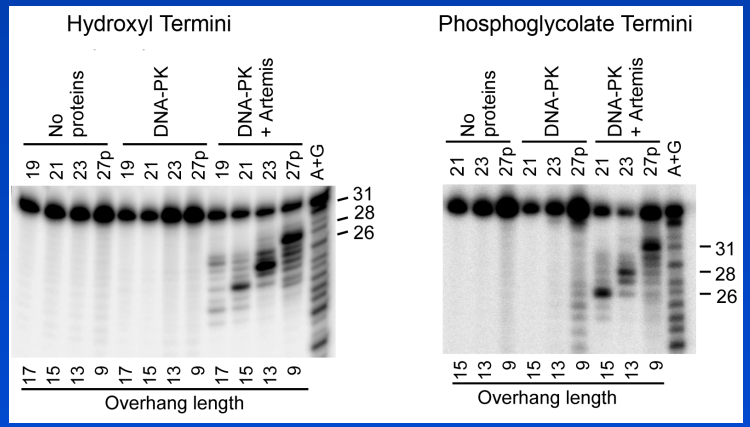
Artemis Efficiently Removes 3' Blocking Groups

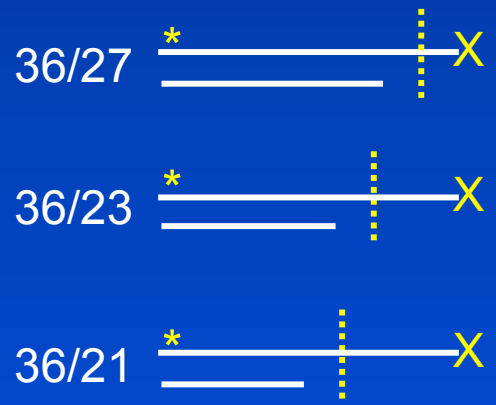
Artemis Registers nuclease on ds/ss DNA transition



Cleavage at ss/ds +5

Artemis can remove blocking moieties by 'bypass'



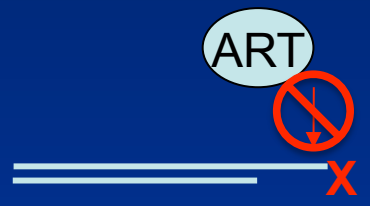


X= -PG or -OH, or P-Tyr

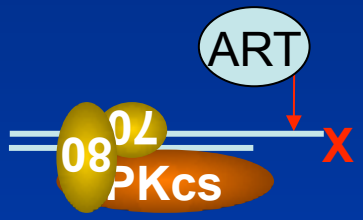
APE1 and TDP1 Very Inefficient

Artemis Biochemical activities

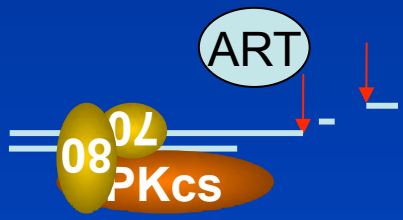
- Artemis activity is strictly dependent on DNA-PK and ATP



- Artemis/DNA-PK can efficiently remove 3' blocking moieties on a 3' overhang via bypass.



- Artemis/DNA-PK is processive and can make multiple cuts



Povirk *et al* .J Biol Chem. 2007 Feb 9;282(6):3547-58.

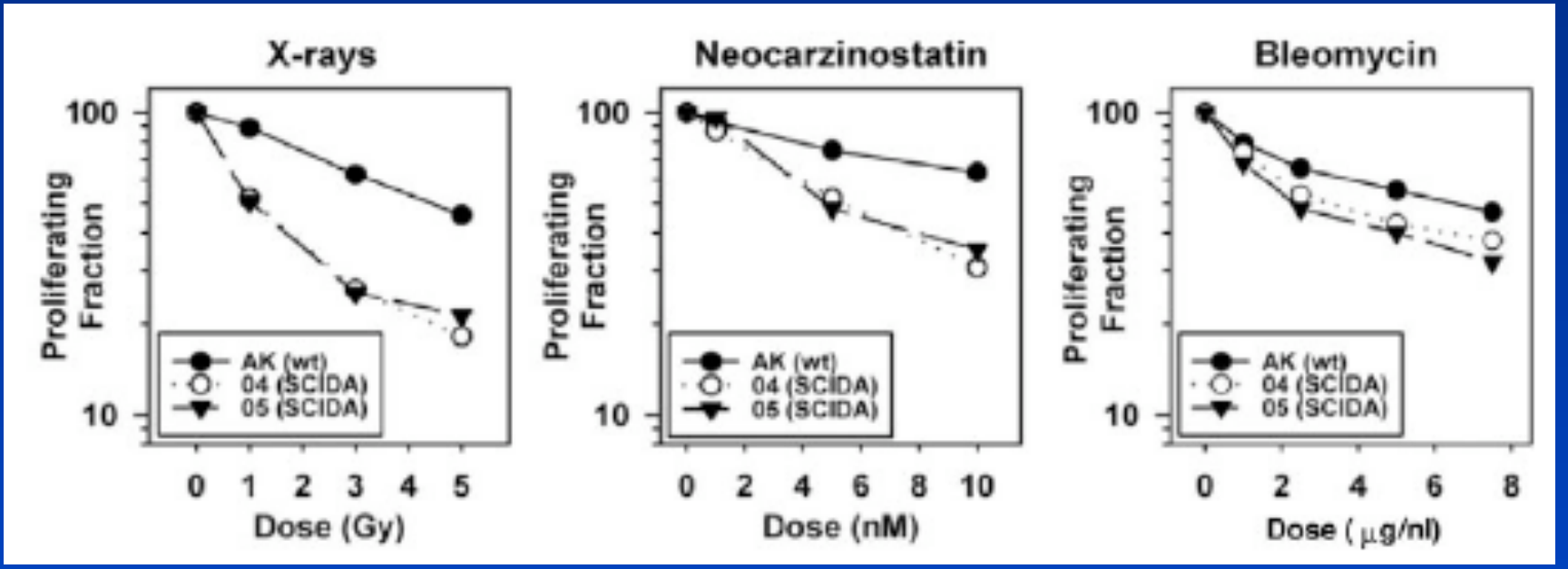
Yannone *et al*,.Nucleic Acids Res. 2008 Jun;36(10):3354-65.

Hypothesis: **Artemis functions to remove 3' blocking groups at DSBs.**

3’ Phosphoglycolate-Inducing Drugs

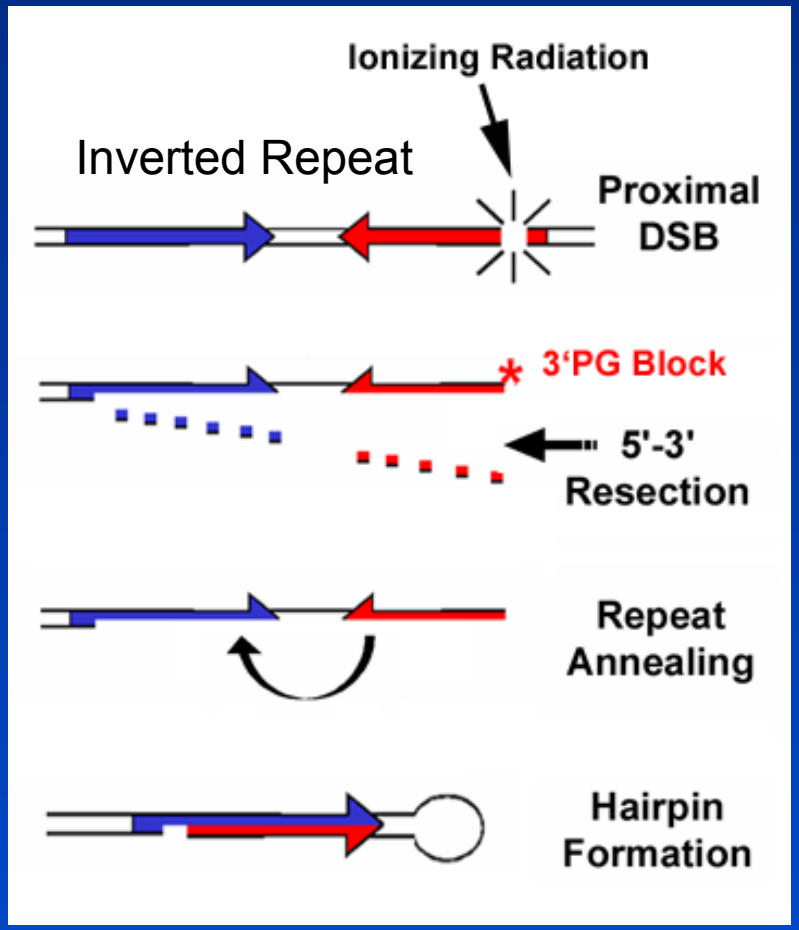
Are Treatments causing more **3’ blocking groups more toxic?**

~ 50% PG **~ 75% PG** **~ 100% PG**



Toxicity does NOT obviously trend with PG induction.

Current Working Hypothesis



Derivative Hairpin Structures

1) Would certainly be a small fraction of total DSBs.

2) Is consistent with Artemis substrate specificity known from V(D)J recombination (non-redundant).

3) Hairpin formation has long been suspected to be the cause of genomic instability arising from inverted repeats.

4) Consistent sequence independence of X-rays as opposed to drugs.

5) Slow 3'-PG removal may encourage hairpin formation by promoting 5'-3' resections.

Take-Home Messages

- Artemis is only active is the context of DNA-PK.
- SCIDA cells are marginally defective in gross DSB repair.
- Artemis functions on a subset of DSBs in the cell.
- Not all DSBs are equally toxic to SCIDA cells.
- Derivative hairpin structures may be an Artemis substrate.

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