DNA Damage Signaling to Immune Checkpoints

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Disclosure

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DNA Damage Signaling to Immune Checkpoints

1) ATR kinase inhibitors induce checkpoint blockade

2) ATR kinase inhibitors induce unscheduled origin firing

Frank Vendetti, PhD

Tatiana Moiseeva, PhD
DNA Damage Signaling

>5,000 substrates

S and G2 phase

double-strand breaks

damaged replication forks

ATMi  ───> ATM  ───> ATR  ───> ATRi

DNA REPAIR
CELL VIABILITY
Inhibitors from Mark O’Connor, PhD

• ATR kinase inhibitor AZD6738 – four clinical trials
  • VX-970 (VE-822) – nine clinical trials

• ATM kinase inhibitor AZ31
  • AZD0156 – one clinical trial
  • AZD1390
ATR kinase inhibition doesn’t kill mice

AZD6738 Pharmacokinetics $t^{1/2} = 6$ h
ATR kinase inhibitor with cisplatin delivers a complete response in ATM-deficient xenografts:
cisplatin before ATR kinase inhibitor
>40% of lung adenocarcinomas are deficient for ATM

Lung SPORE TMA

>200 cases were included in the analysis. Inclusion criteria were +ve staining in stroma and immune cell infiltrate, and at least 2 cores scored for each patient.

Villaruz L (2016) Oncotarget
Clinical and preclinical studies with ATR kinase inhibitors are justified
ATM kinase inhibitor sensitizes mice to total body irradiation
ATR kinase inhibitor before radiation

Vendetti F (2017) Scientific Reports
ATM kinase inhibitor with radiation kills intestinal crypts
Genetically engineered mouse model of NSCLC

CCSP-rtTA
tetO-Kras$^{G12D}$
Luc-tetO-Twist1

Small Animal Radiation Research Platform (SARRP)

Frank Vendetti, PhD
ATR kinase inhibitor with radiation delivers a delayed response

ATR kinase inhibitor before radiation

ATRi + IR
IR (3 Gy x10)
ATRi
Vehicle

Percent Tumor Growth

Day

end of treatment
delayed response
ATM kinase inhibitor with radiation kills hair follicles
The impact of ATR kinase inhibitor is CD8$^+$ T cell dependent
Immunology

1) Radiation
2) Antigen Release
3) Antigen Cross Presentation
4) Anti-Tumor Immune Responses

Adaptation by A Sharabi, original figure from Sharabi A et al. (2016) Oncology, (Williston Park) 29(5)

Adapted from Pardoll DM. (2012) Nat Rev Cancer, 12(4)
ATR kinase inhibitor potentiates radiation in syngeneic CT26 colorectal carcinomas

ATR kinase inhibitor before radiation
ATR kinase inhibitor attenuates radiation-induced PD-L1 upregulation \textit{in vivo}

-ISotype Vehicle IR ATRi + IR-

Day 5

![Day 5 Graph]

**Fold change MFI**
ATR kinase inhibitor blocks T cell exhaustion after radiation
ATR kinase inhibitor induces checkpoint blockade after radiation

cytokine production following PMA/ionomycin
Kinase inhibition does not phenocopy kinase knockout

>5,000 substrates
Molecular Mechanism

- Cell intrinsic signaling - radiation
  - cGAS-STING knockout in CT26

- Cell extrinsic signaling - interferon $\gamma$

- Cancer cells - cell cycle checkpoint competent?
  - Lung cancer patients will have been treated with platinum-based chemotherapy combinations
    - Leonna Samson, PhD
    - Trey Ideker, PhD and Mike Yaffe, MD, PhD

- Stromal cells
- Immune cells
  - CD8$^+$ T cells and Tregs
ATR kinase inhibitor induces origin firing in undamaged cells

Couch et al. Cortez D. Genes Dev. 2013
Kwok et al. Stankovic T. Blood 2016
ATR kinase inhibitor induces an association of replisome components in chromatin

Moiseeva et al. (2017) Nature Communications
ATR and Chk1 kinase inhibitors induce Cdc7-dependent MCM4 hyper-phosphorylation
ATR kinase inhibitor induces MCM4 hyper-phosphorylation in S phase cells
ATR kinase inhibitor induces Cdc7-dependent MCM4 hyper-phosphorylation
DNA damage before ATR kinase inhibitor changes the signaling
DNA damage before ATR kinase inhibitor changes the signaling
DNA damage before ATR kinase inhibitor changes the signaling.
ATR kinase inhibitor induces origin firing and this leads to more DSBs when H$_2$O$_2$ is added.
Summary

Sequence is important
ATR kinase inhibitor induces an association of replisome components in chromatin

Moiseeva et al. (2017) Nature Communications
Ctf4/And-1 in yeast
WHDH1/And-1 in humans

A Ctf4 trimer couples the CMG helicase to DNA polymerase α in the eukaryotic replisome.

ATR and Chk1 kinase inhibitors induce the association of GINS, And-1 and DNA pol α.
ATR kinase inhibitor induces Cdc7-dependent phosphorylation on GINS4
Phosphorylated S12/S16 GINS associates with C terminus of And-1
Summary

Inhibitors of DNA damage signaling are not inert
PBMCs do not express ATR

Bakkenist CJ et al. (2015) Oncoscience
T cell expansion ex vitro

dye is diluted as T cells divide

CD8+ no activation

anti-CD3 + anti-CD28 + IL-2 (100U/mL)
dye is diluted as T cells divide
ATR kinase inhibitor blocks T cell expansion *ex vivo*

![Flow cytometry plot showing CD8+ and CD44 expression with CFSE staining.](image)

ATR kinase inhibitors block T cell expansion

*ATR kinase inhibitor blocks T cell expansion*
It is important to identify the structures generating change within the complexity.

Search for ATR kinase inhibitors generates 2,500 hits on Pubmed.
Sequence is important

- If the cancer is cell cycle checkpoint deficient, genotoxic chemotherapy before ATR kinase inhibitor

- If the cancer is DNA repair deficient, ATR kinase inhibitor and then genotoxic chemotherapy

- The ATR kinase inhibitor must be removed to allow an anti-tumor immune response
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