

DNA Damage Signaling to Immune Checkpoints

Chris Bakkenist, PhD, Associate Professor
Department of Radiation Oncology,
UPMC Hillman Cancer Center
University of Pittsburgh



Disclosure

The Bakkenist Lab has sponsored research agreements with AstraZeneca

DNA Damage Signaling to Immune Checkpoints

1) ATR kinase inhibitors induce checkpoint blockade



Frank Vendetti, PhD

2) ATR kinase inhibitors induce unscheduled origin firing

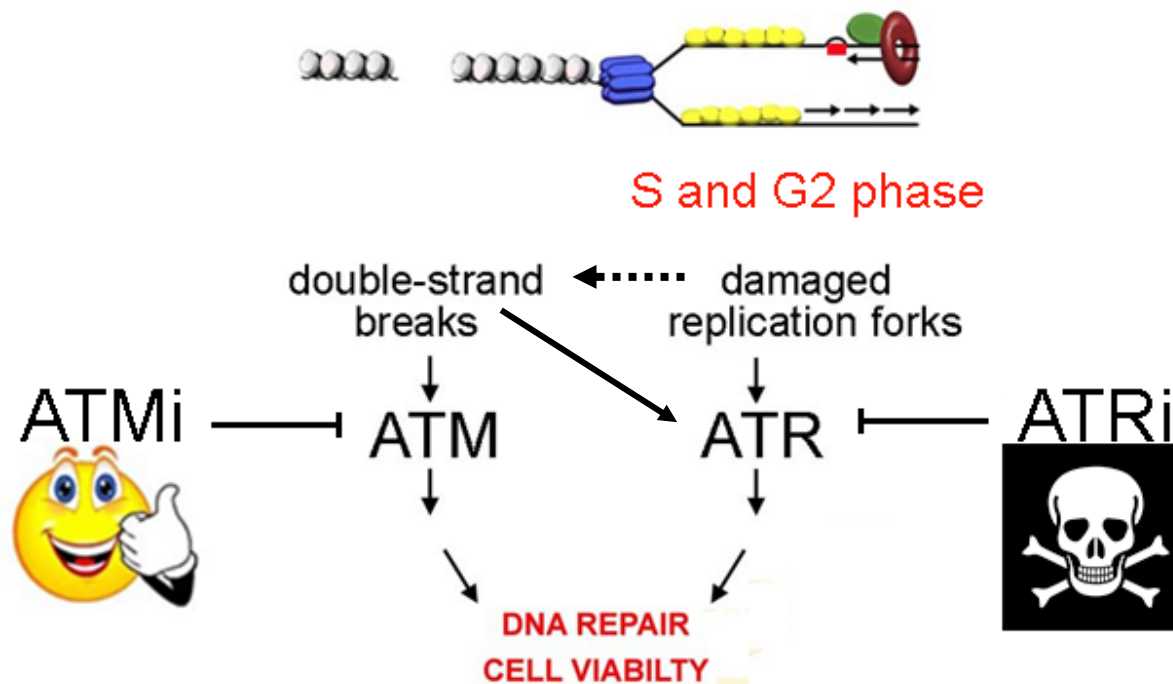


Tatiana Moiseeva, PhD

DNA Damage Signaling

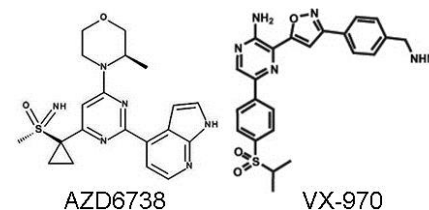


>5,000 substrates



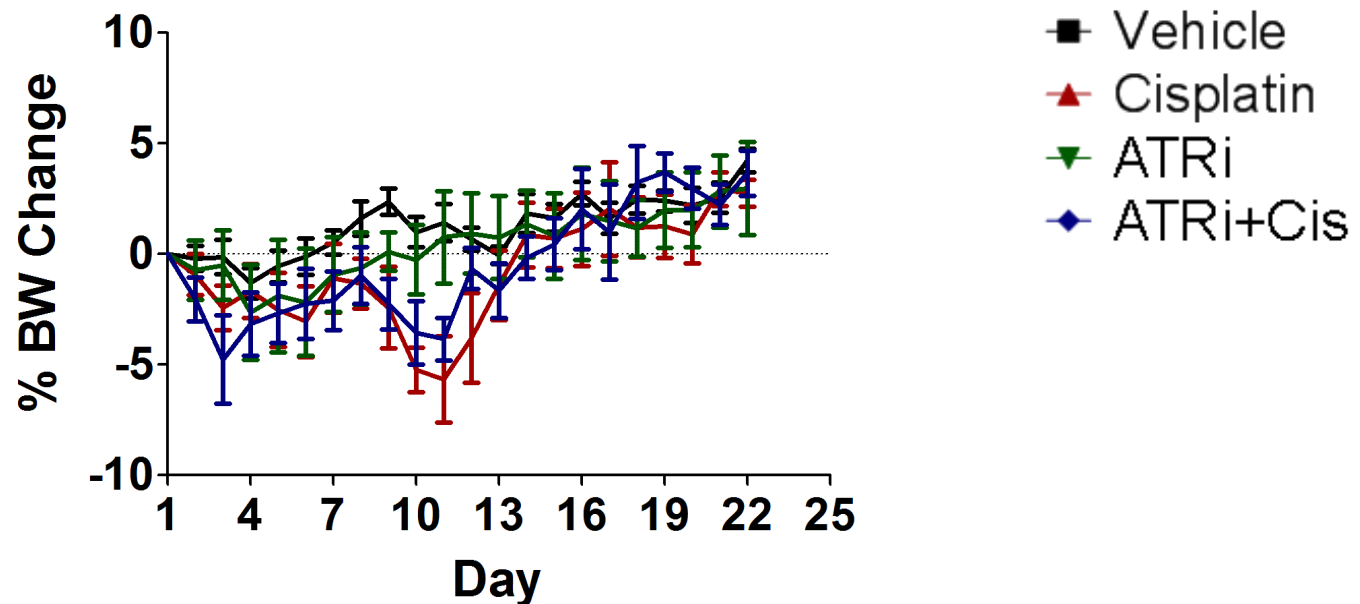
Inhibitors from Mark O'Connor, PhD

- 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

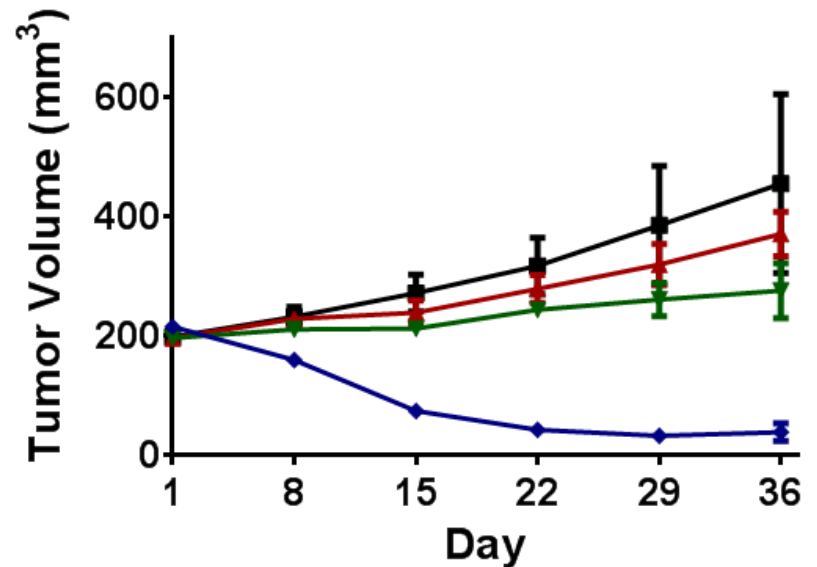
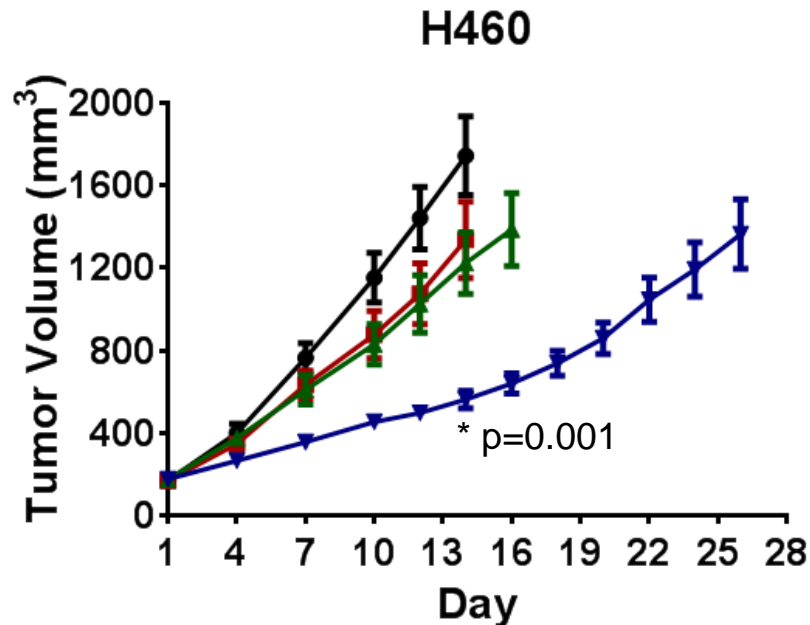


AZD6738 Pharmacokinetics $t_{1/2} = 6$ h

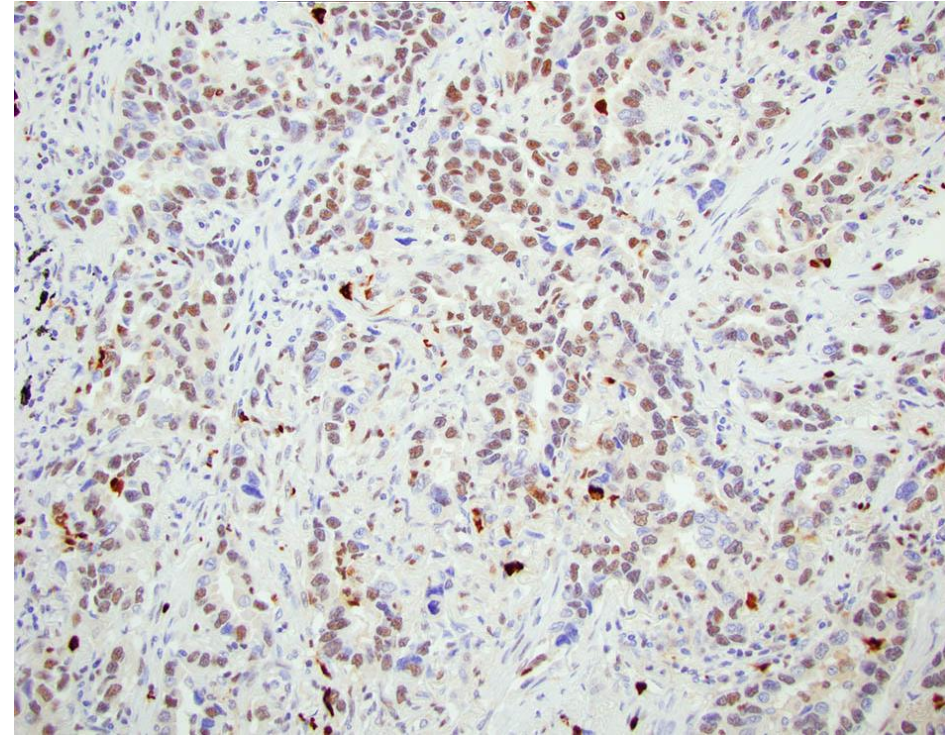
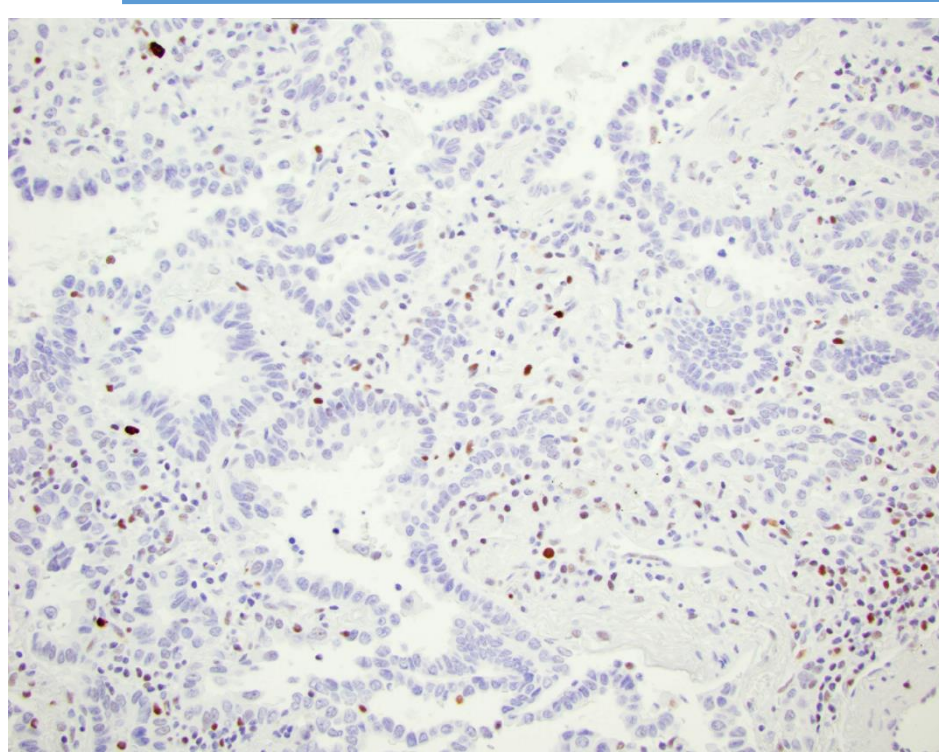
ATR kinase inhibitor with cisplatin delivers a complete response in ATM-deficient xenografts

cisplatin before ATR kinase inhibitor

ATM-deficient



>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.

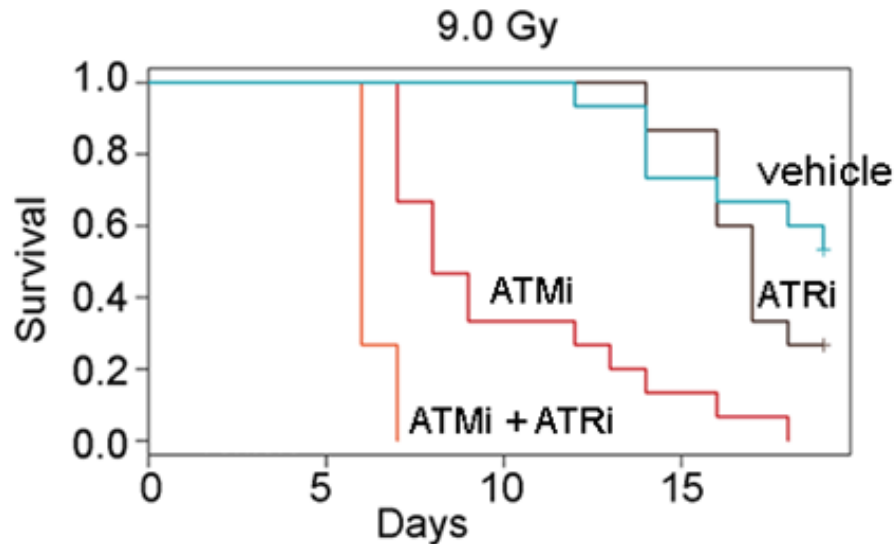
Villaruz L (2016) Oncotarget

Summary

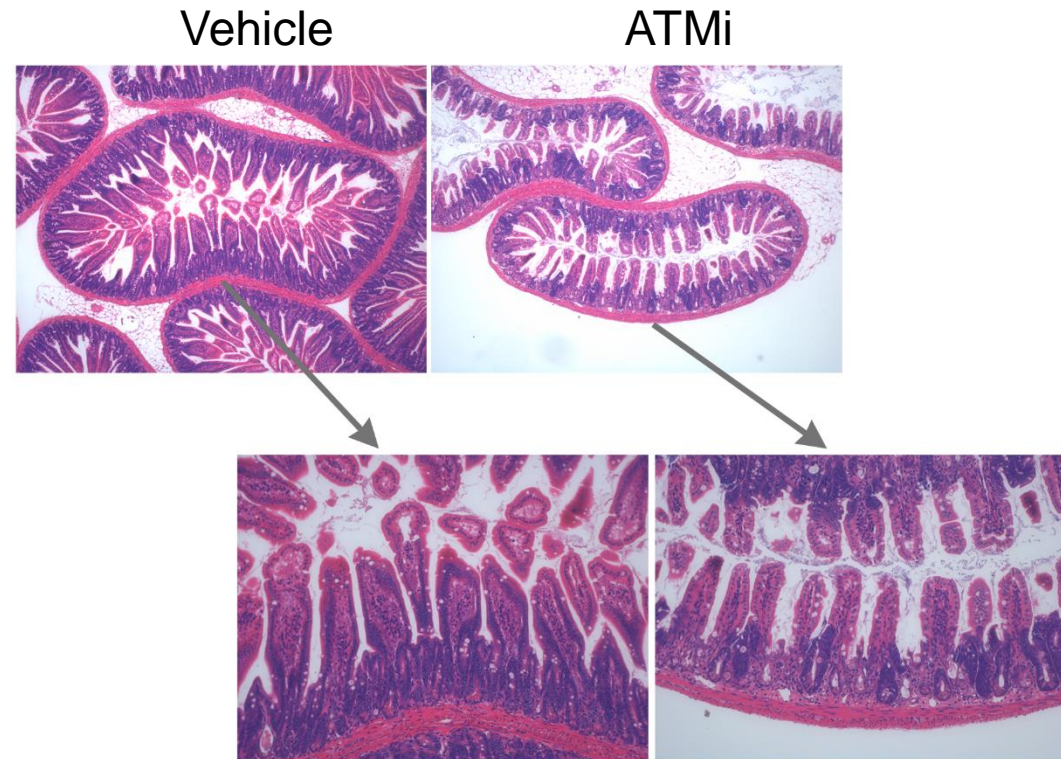
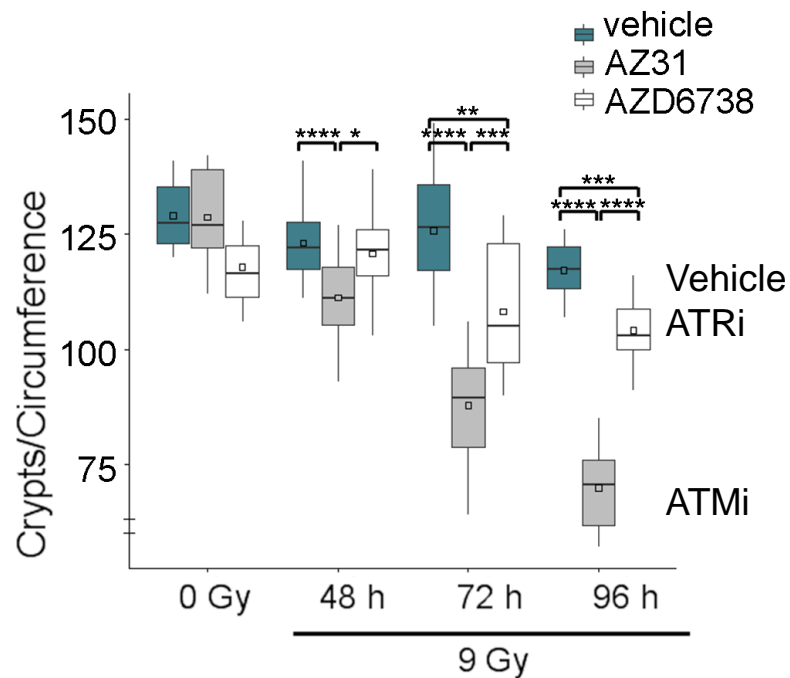
Clinical and preclinical studies with ATR kinase inhibitors are justified

ATM kinase inhibitor sensitizes mice to total body irradiation

ATR kinase inhibitor before radiation



ATM kinase inhibitor with radiation kills intestinal crypts



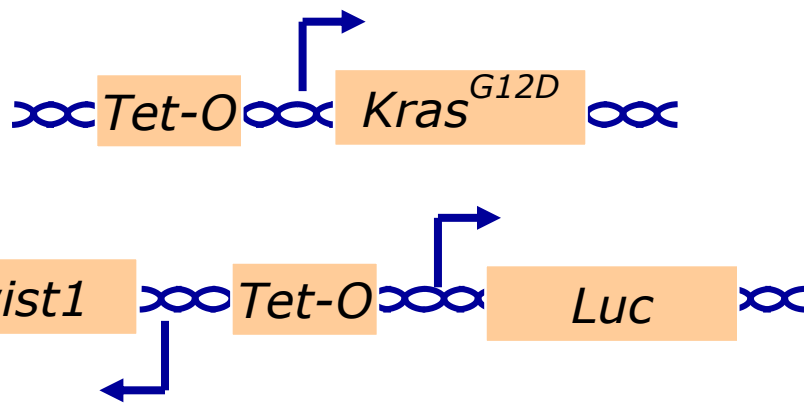
Genetically engineered mouse model of NSCLC



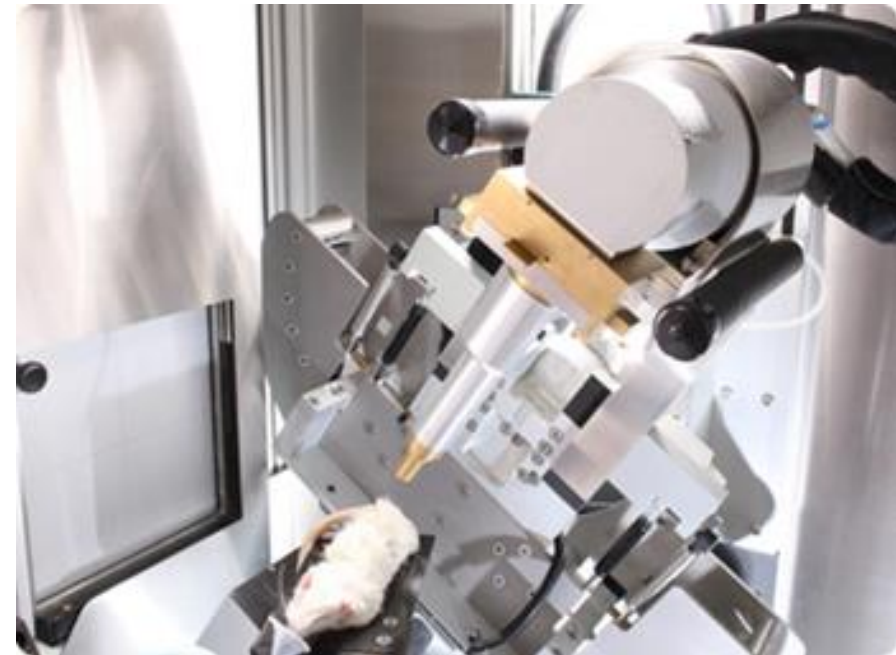
Frank Vendetti, PhD



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

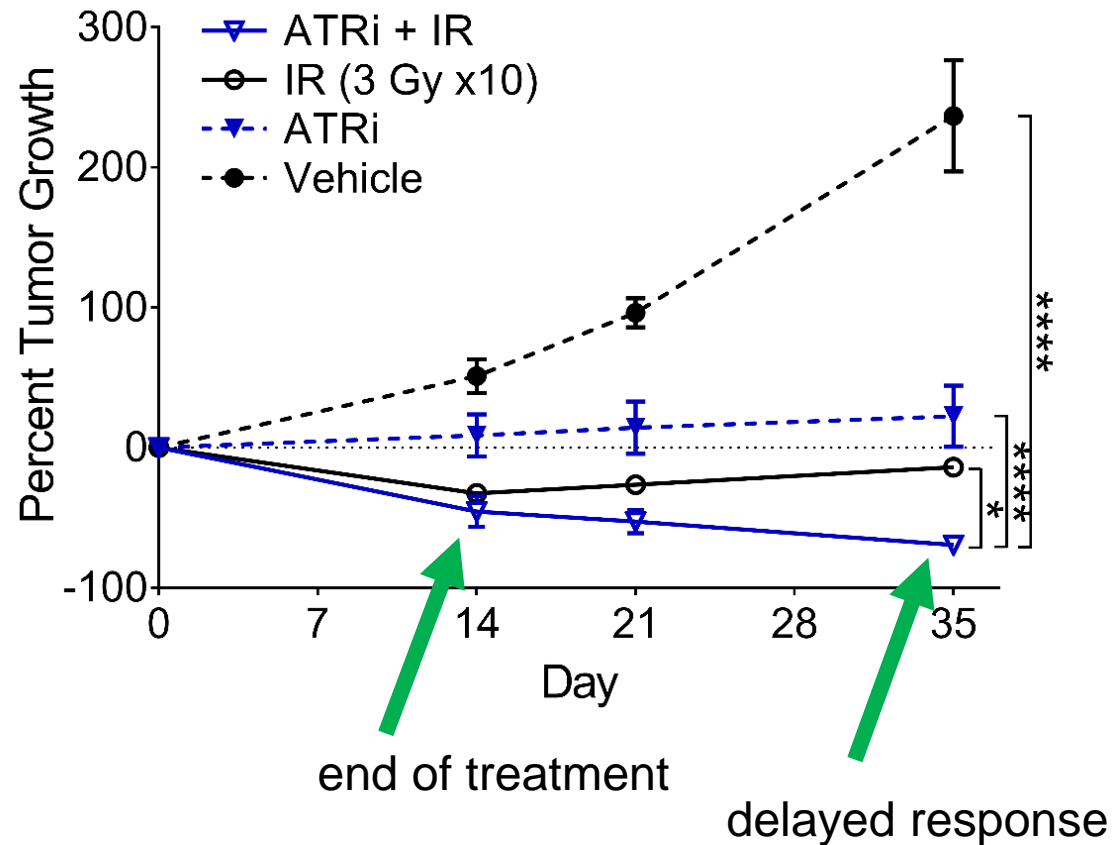
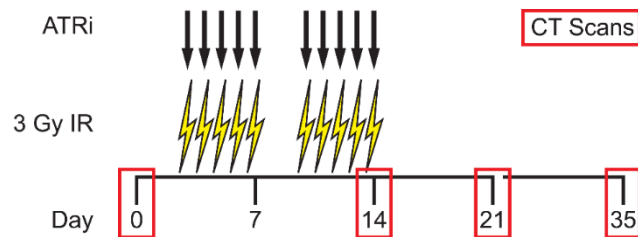


Small Animal Radiation Research
Platform (SARRP)

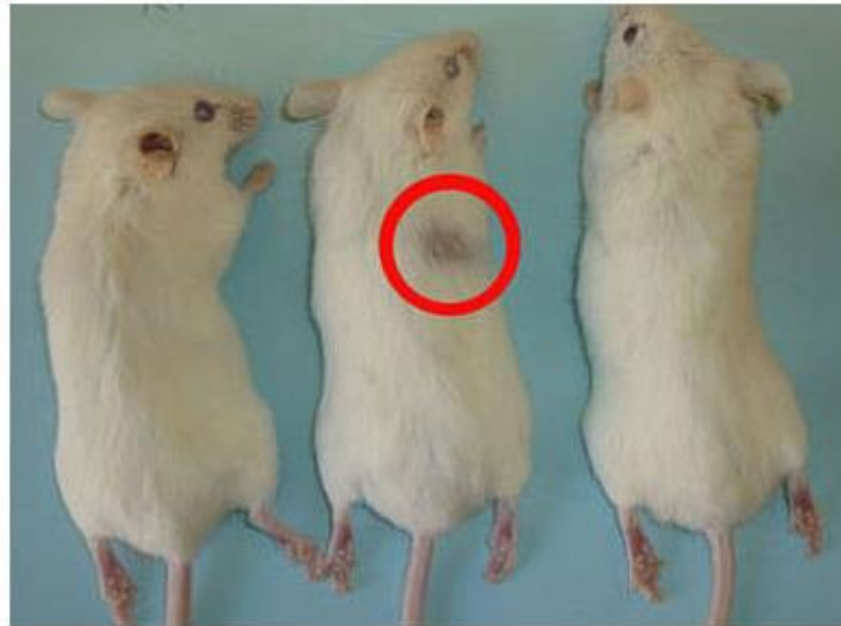


ATR kinase inhibitor with radiation delivers a delayed response

ATR kinase inhibitor before radiation



ATM kinase inhibitor with radiation kills hair follicles

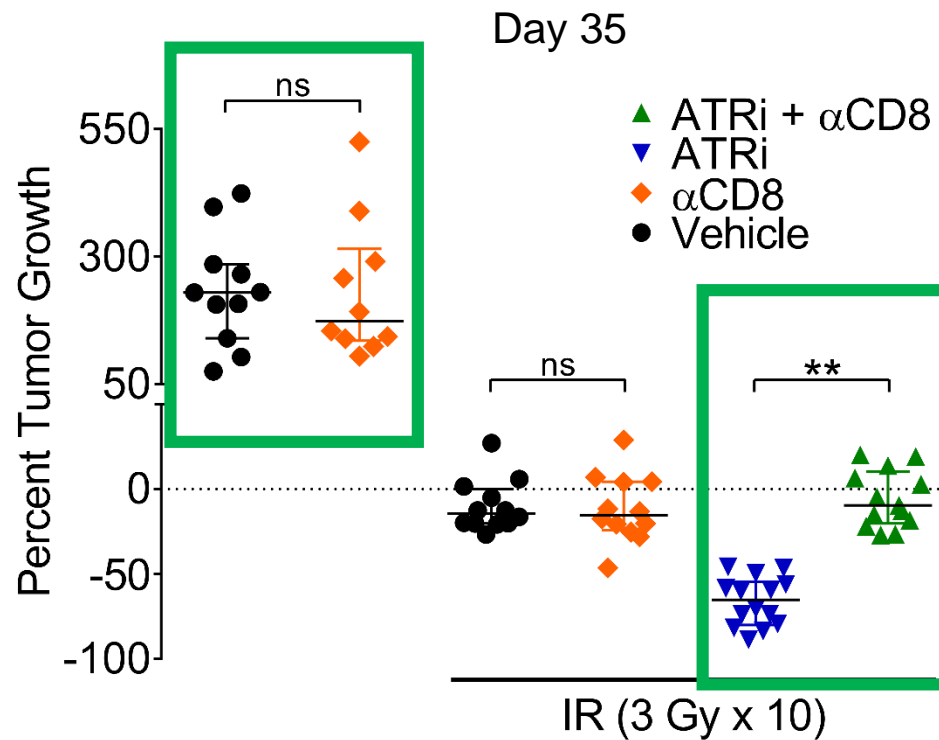


veh

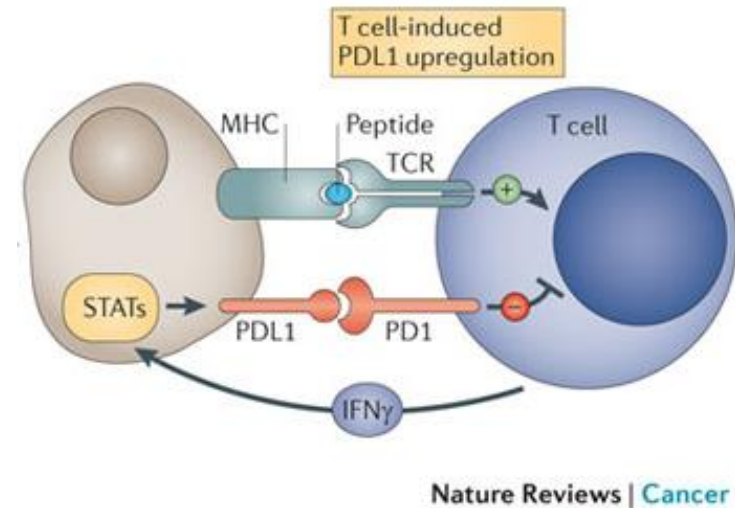
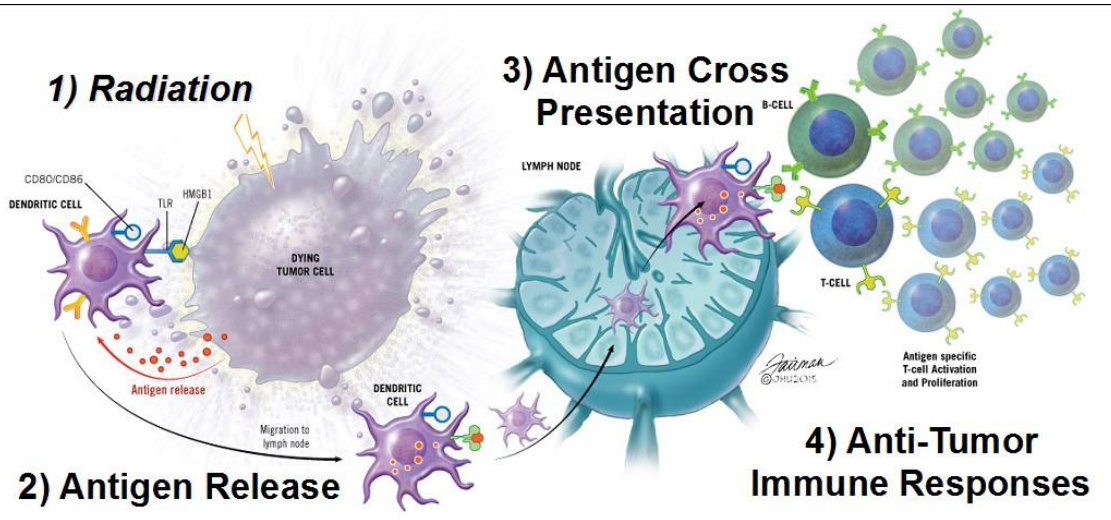
ATMi

ATRi

The impact of ATR kinase inhibitor is CD8⁺ T cell dependent

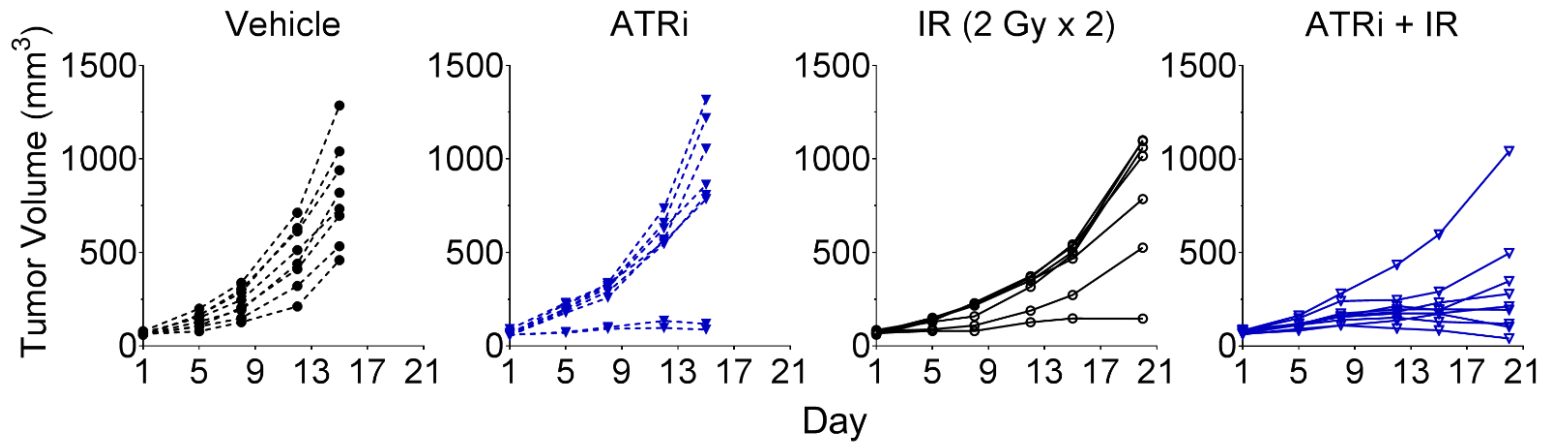
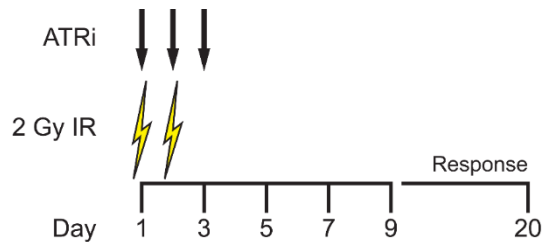


Immunology

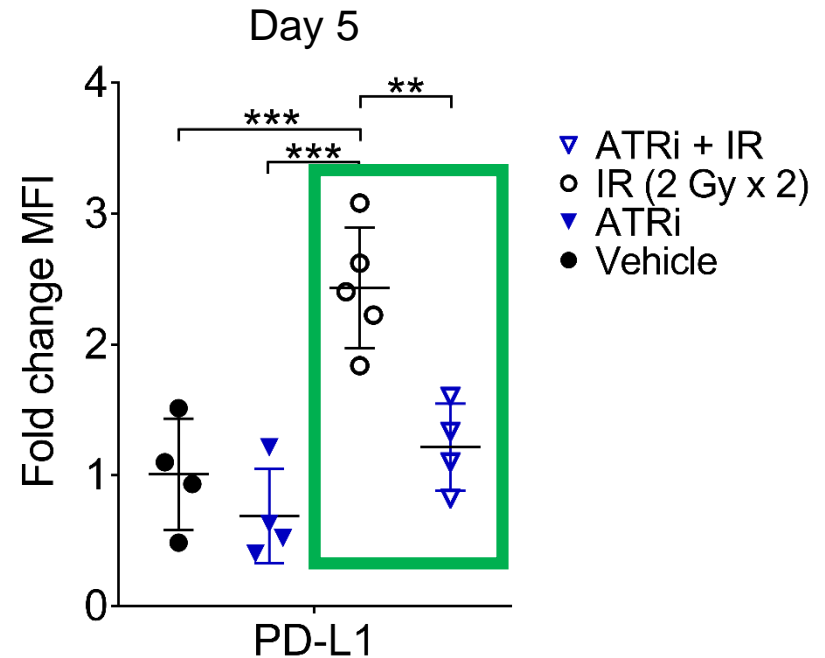
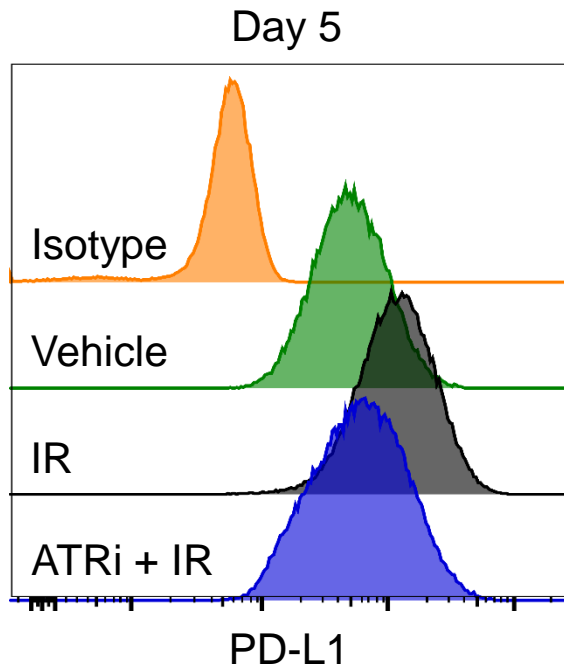
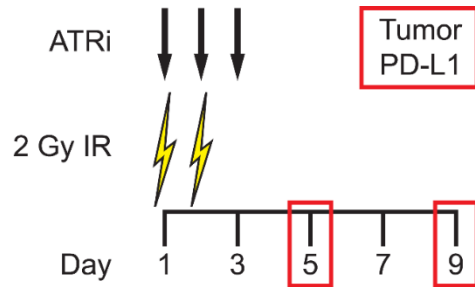


ATR kinase inhibitor potentiates radiation in syngeneic CT26 colorectal carcinomas

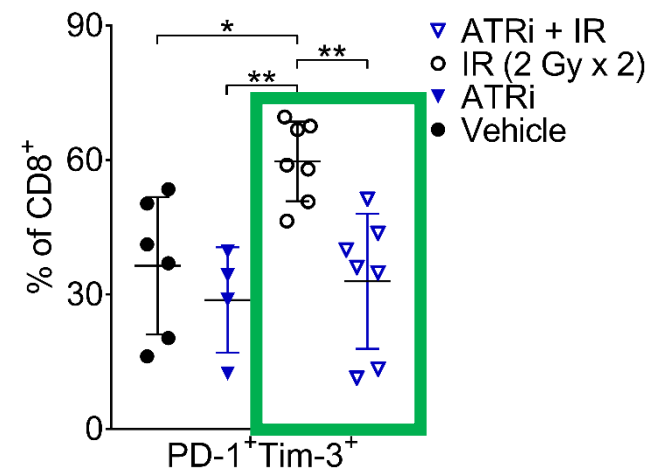
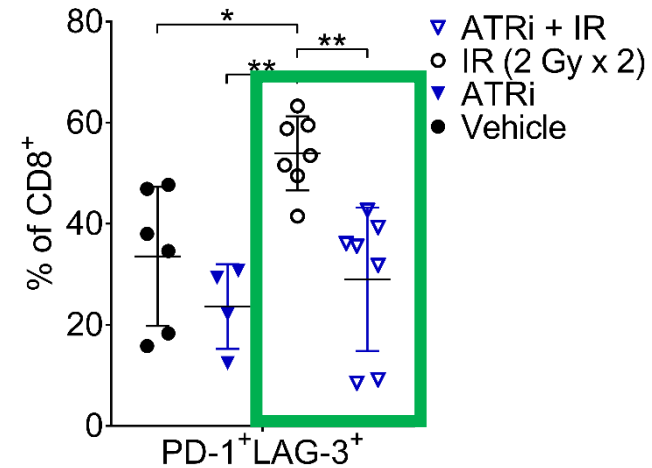
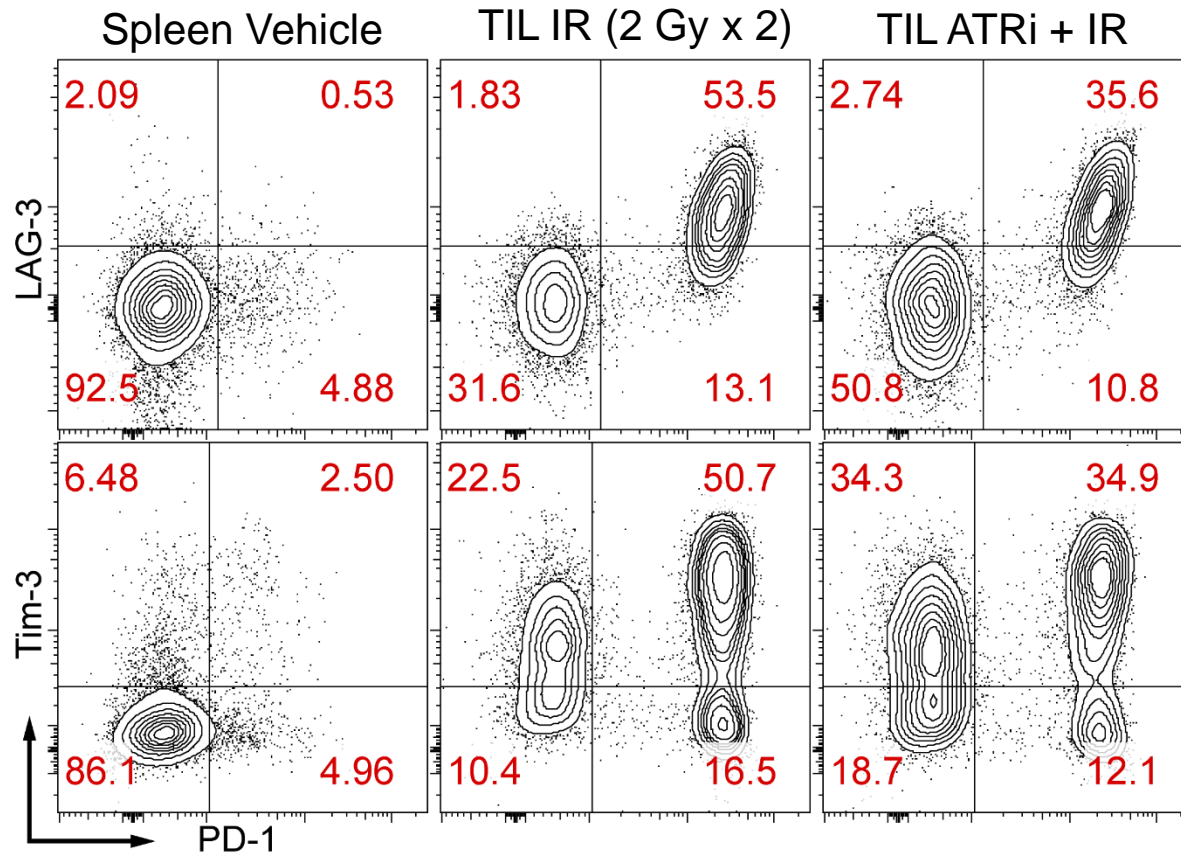
ATR kinase inhibitor before radiation



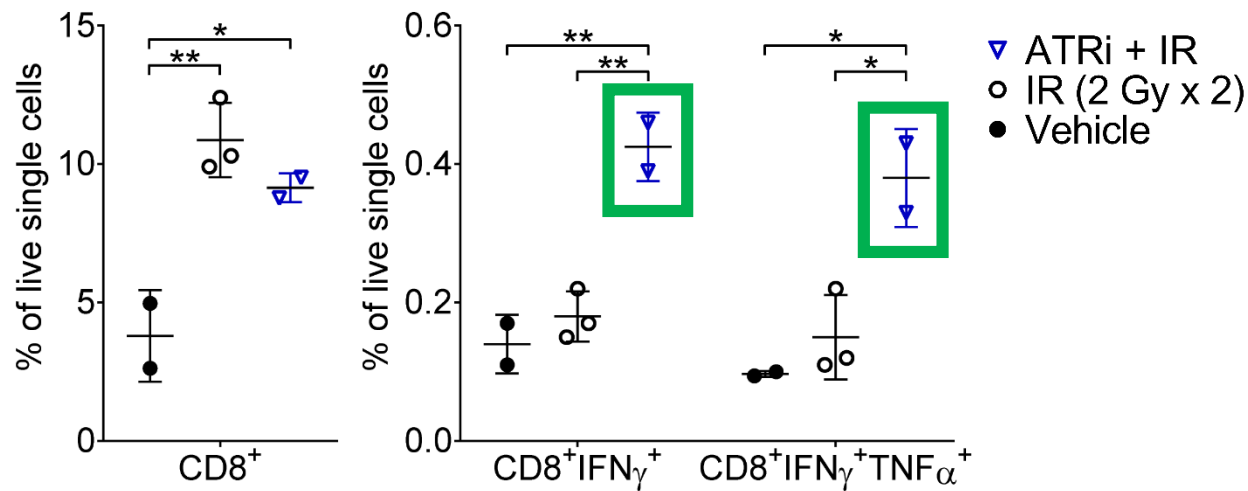
ATR kinase inhibitor attenuates radiation-induced PD-L1 upregulation *in vivo*



ATR kinase inhibitor blocks T cell exhaustion after radiation



ATR kinase inhibitor induces checkpoint blockade after radiation

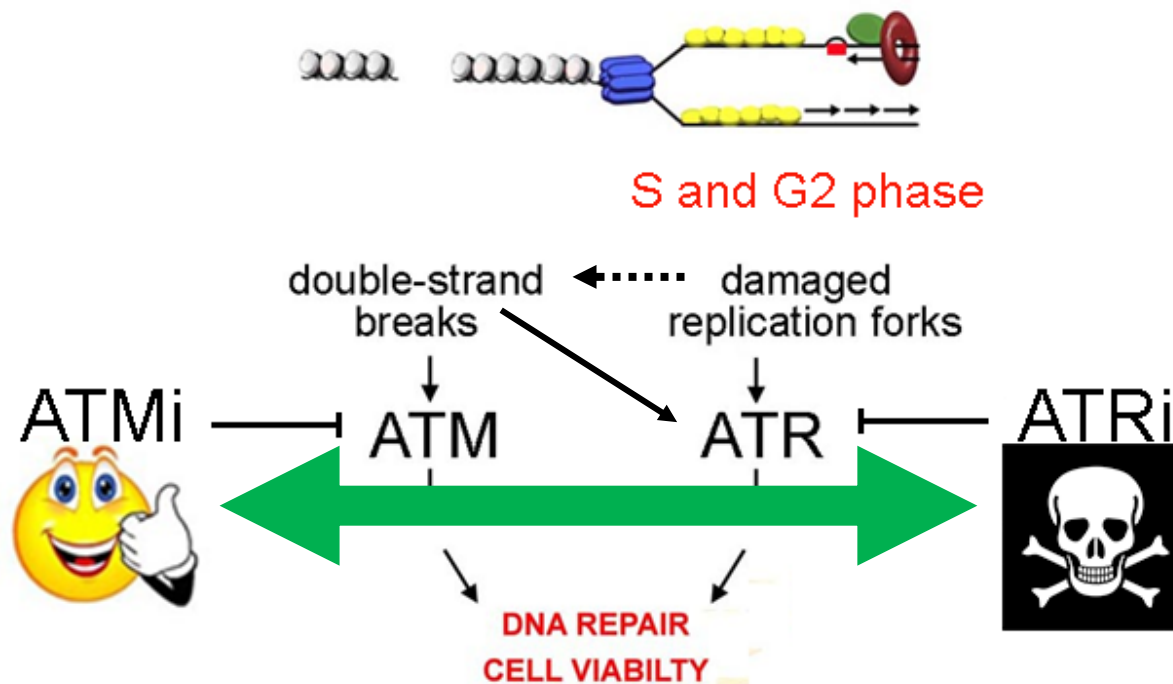


cytokine production
following PMA/ionomycin

Kinase inhibition does not phenocopy kinase knockout



>5,000 substrates



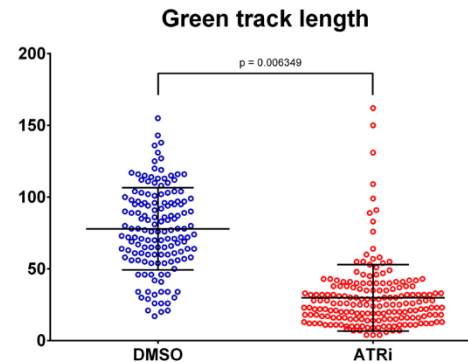
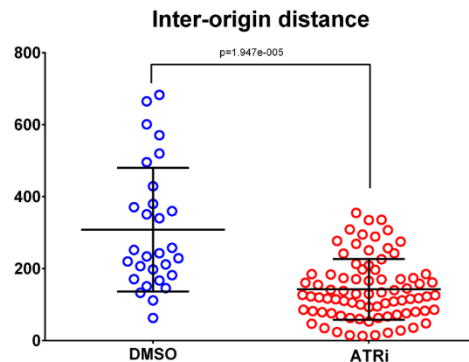
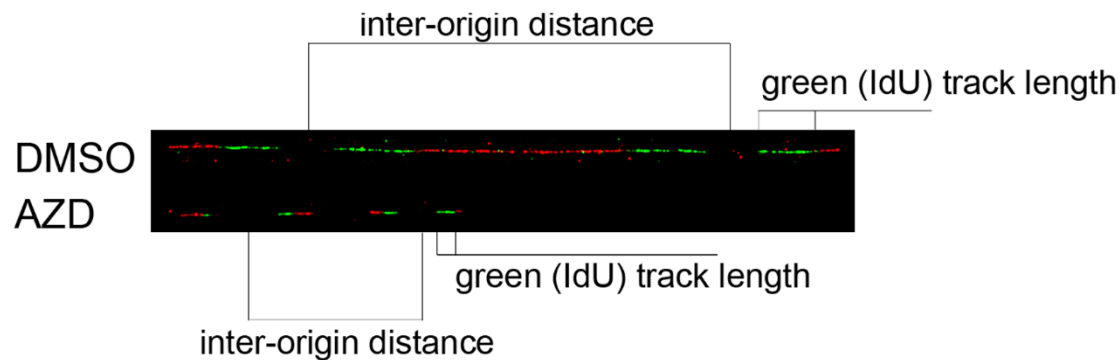
Molecular Mechanism

- Cell intrinsic signaling - radiation
 - cGAS-STING knockout in CT26
- Cell extrinsic signaling - interferon γ
- 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

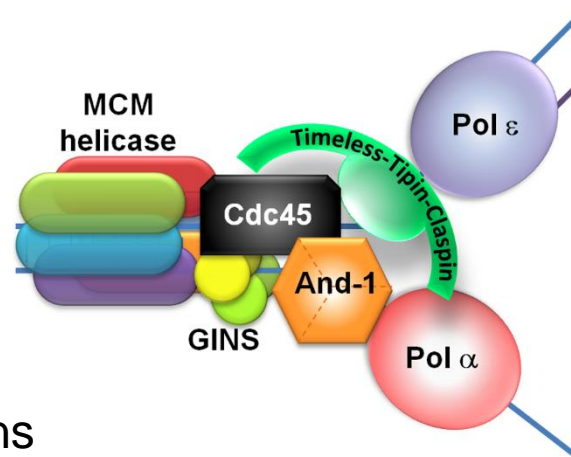
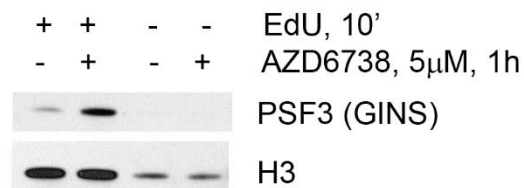
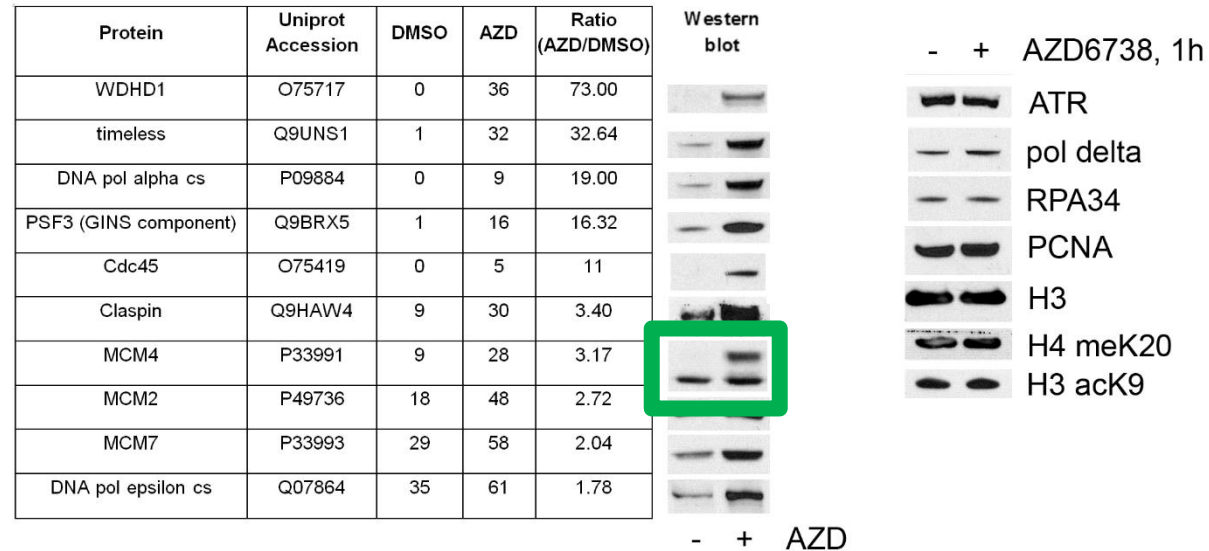


Tatiana Moiseeva, PhD

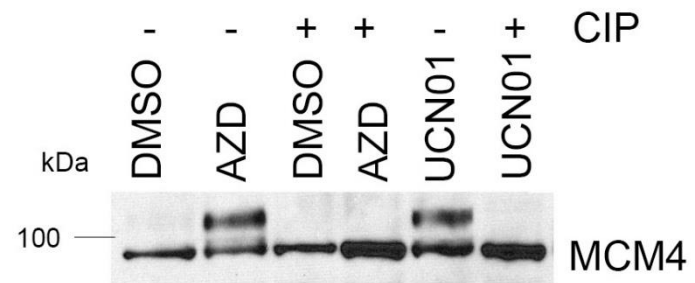
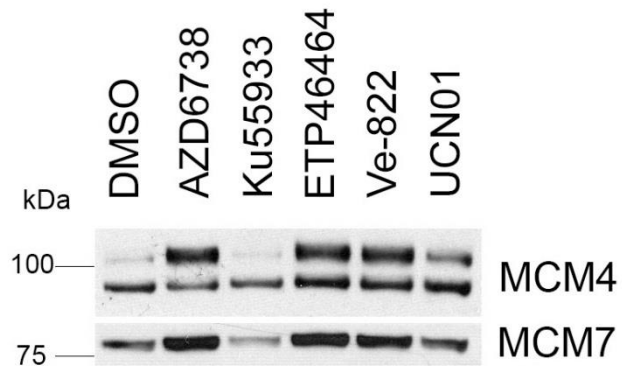


Couch et al. Cortez D. Genes Dev. 2013
Kwok et al. Stankovic T. Blood 2016

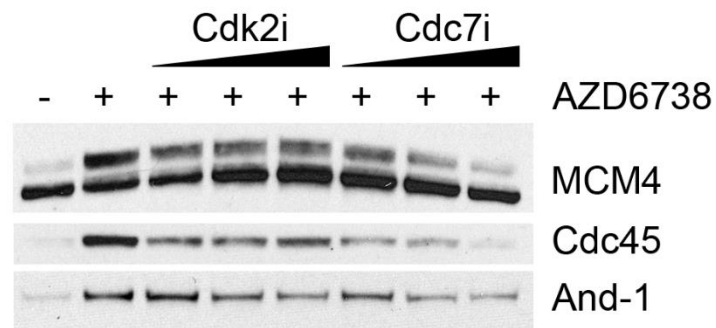
ATR kinase inhibitor induces an association of replisome components in chromatin



ATR and Chk1 kinase inhibitors induce Cdc7-dependent MCM4 hyper-phosphorylation



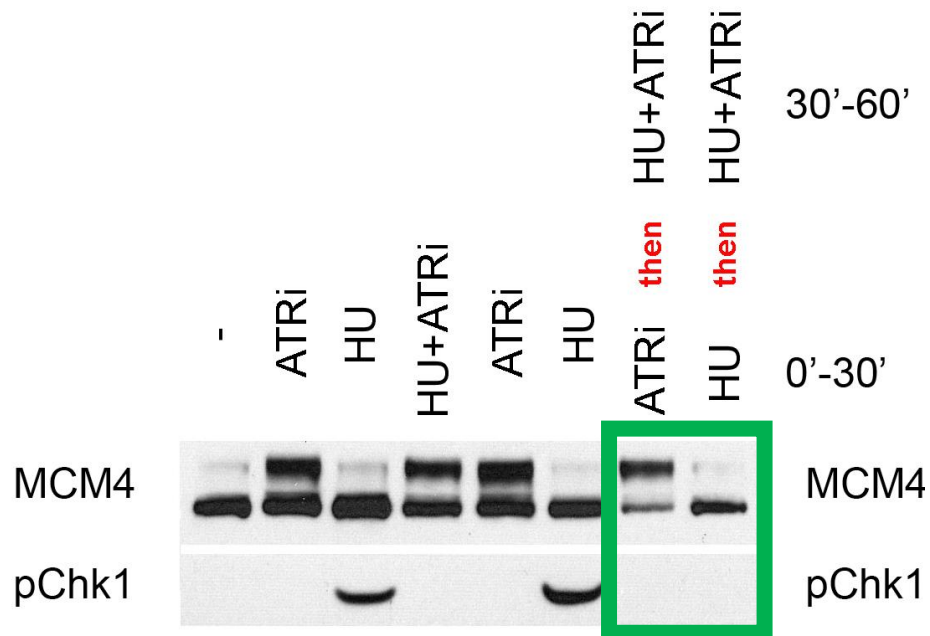
ATR kinase inhibitor induces Cdc7-dependent MCM4 hyper-phosphorylation



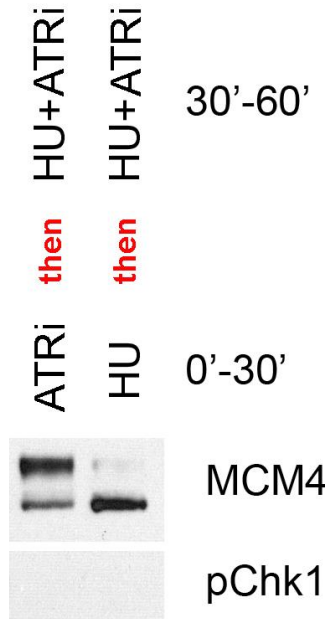
ATR kinase —| Cdc7 kinase

ATRi —→ Cdc7 kinase ↑↑↑

DNA damage before ATR kinase inhibitor changes the signaling



DNA damage before ATR kinase inhibitor changes the signaling



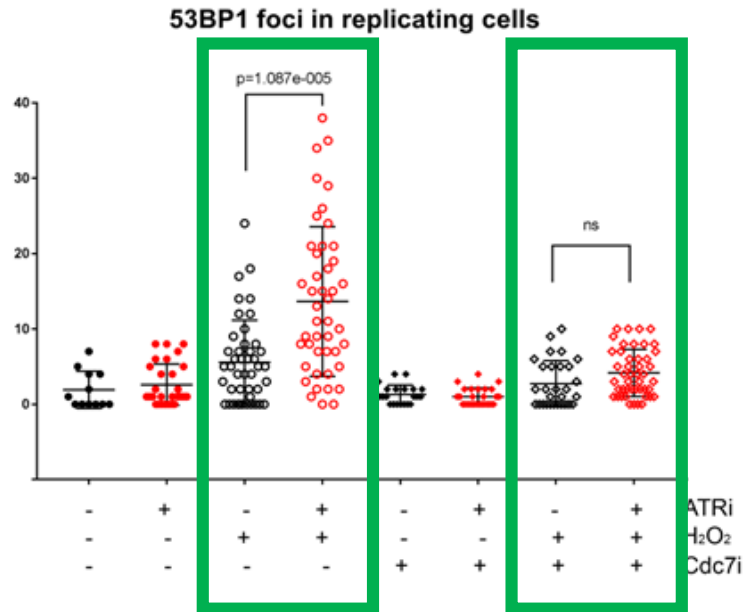
ATR kinase  Cdc7 kinase

ATRi  Cdc7 kinase 

ATR kinase  ATRi  Cdc7 kinase



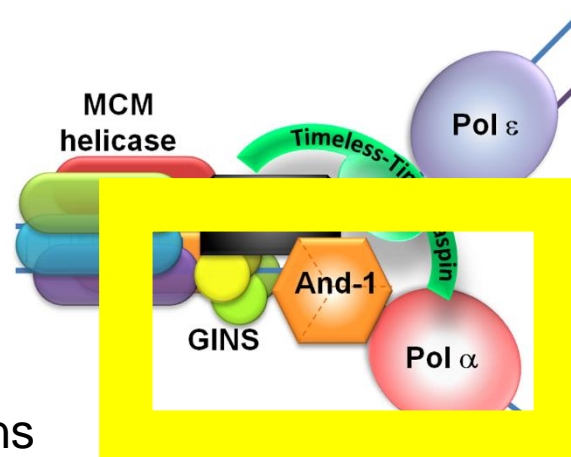
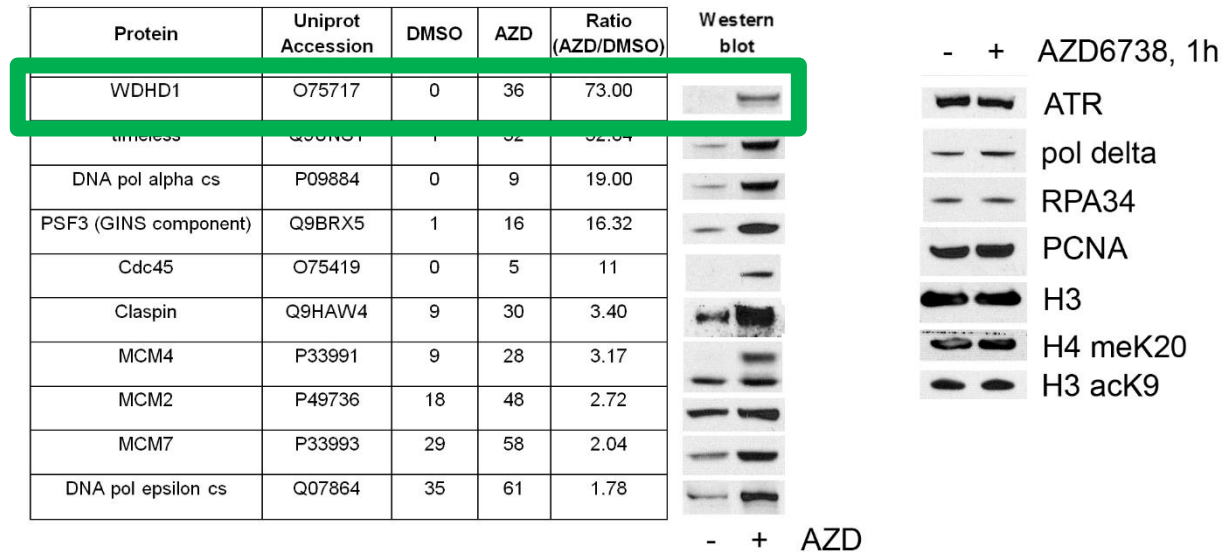
ATR kinase inhibitor induces origin firing and this leads to more DSBs when H₂O₂ is added



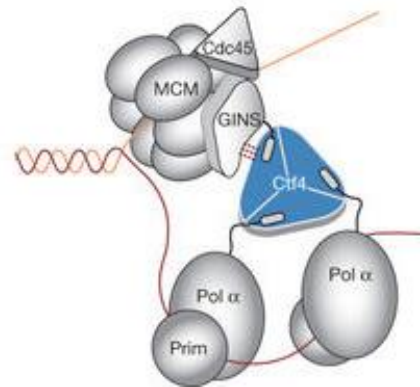
Summary

Sequence is important

ATR kinase inhibitor induces an association of replisome components in chromatin



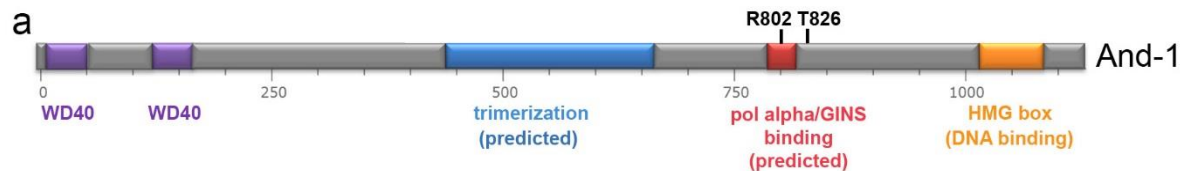
Ctf4/And-1 in yeast WHDH1/And-1 in humans



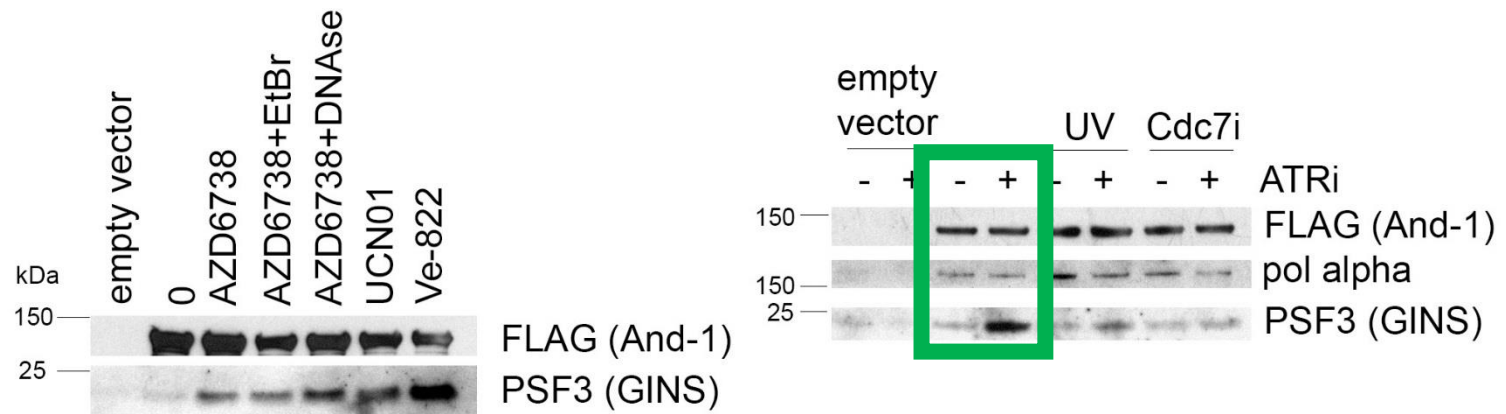
Simon.....Pellegrini

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

Nature 510, 293-297 (2014)

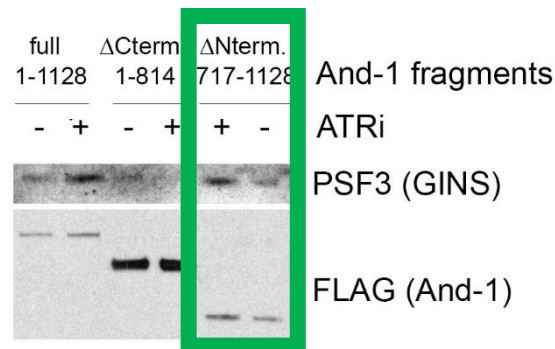
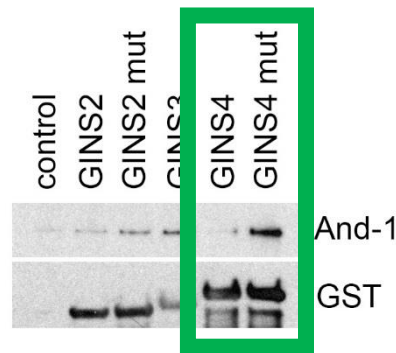


ATR and Chk1 kinase inhibitors induce the association of GINS, And-1 and DNA pol α



ATR kinase inhibitor induces Cdc7-dependent phosphorylation on GINS4

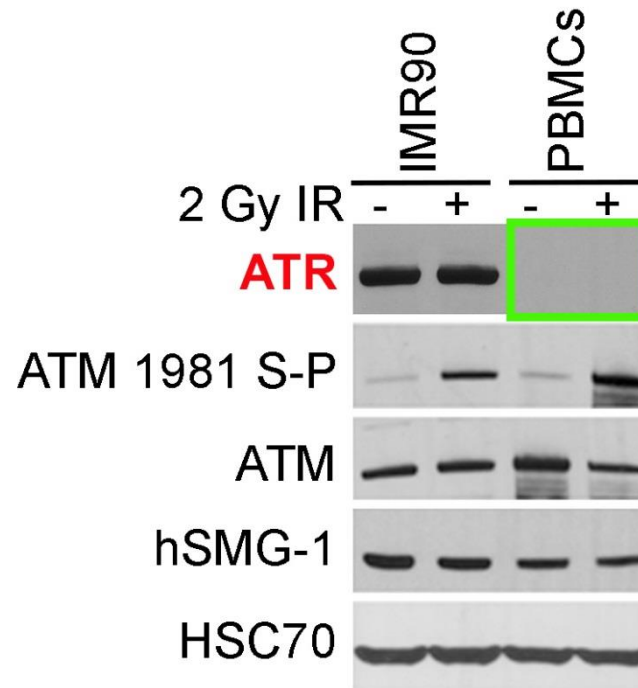
Phosphorylated S12/S16 GINS associates with C terminus of And-1



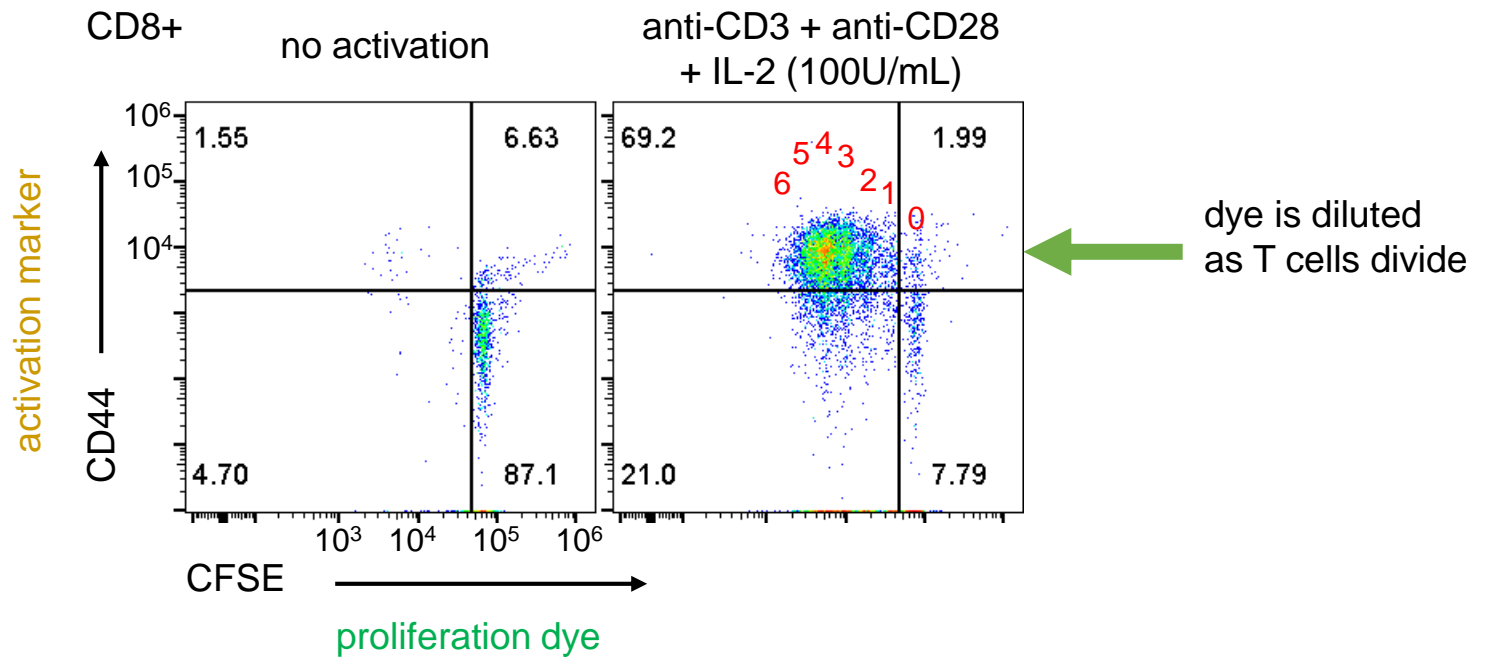
Summary

Inhibitors of DNA damage signaling are not inert

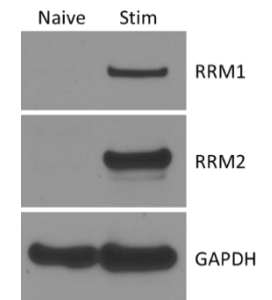
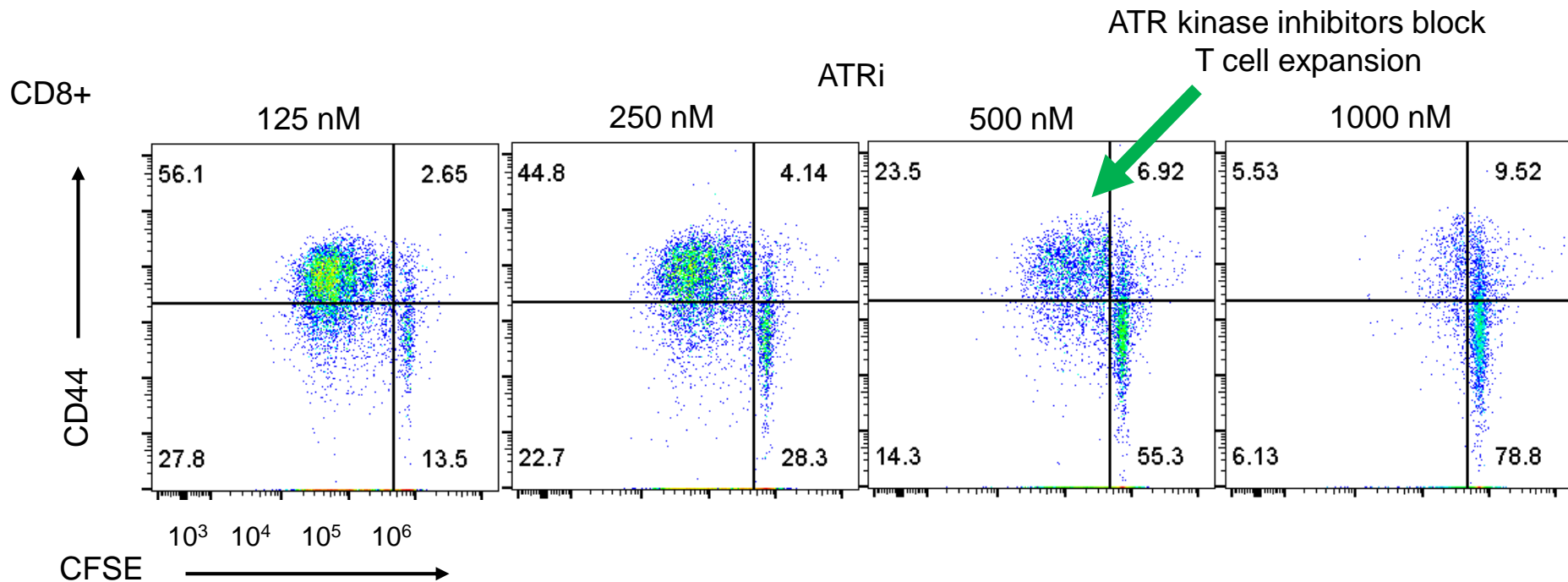
PBMCs do not express ATR



T cell expansion *ex vitro*



ATR kinase inhibitor blocks T cell expansion *ex vitro*



It is important to identify the structures generating change within the complexity

Cancer

Immune System



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

Acknowledgements

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NATIONAL
CANCER
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- Saumen Sarkar

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- Jennifer Barnes
- Elaine Barnes
- Andrew Pierce

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- Phuoc Tran

AstraZeneca 

Always have openings
for new postdocs

