CHK1 inhibitor combination induces multiple cell death forms in melanomas and modifies the tumour immune microenvironment

Zhen Zeng [1*], Rituparna Bhatt [1], Si En Ong [1], Martina Proctor [1], Jazmina L. Gonzalez Cruz [2], Katharine Irvine [1], Riccardo Dolcetti [3], James W. Wells [2], Brian Gabrielli [1]

[1] Mater Research Institute, The University of Queensland, Brisbane, QLD 4102, Australia; [2] Frazer Institute, The University of Queensland, Brisbane, QLD 4102, Australia; [3] Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia

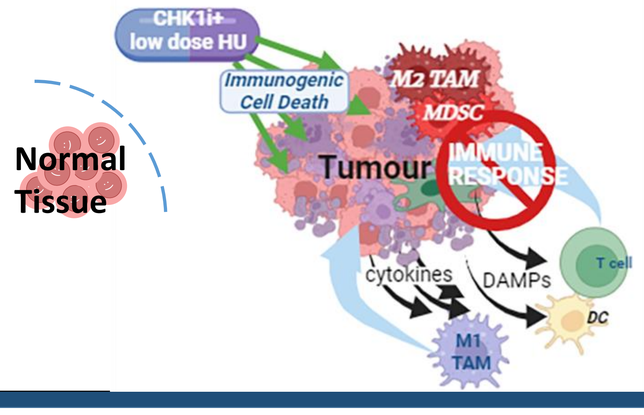
THE UNIVERSITY OF QUEENSLAND AUSTRALIA

*Presenter contact: jenny.zeng@mater.uq.edu.au

mater research

Background

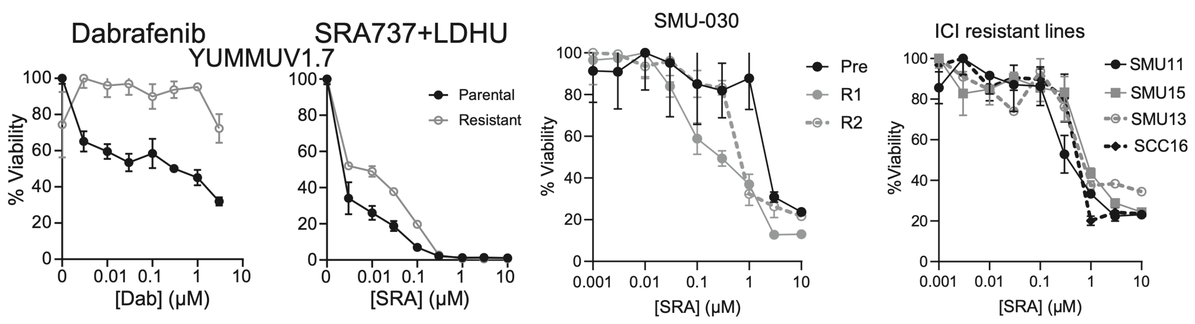
- Replication stress is a common feature in most cancers, including melanoma and high grade serious ovarian cancer
- Can effectively target replication stress using Checkpoint kinase 1 inhibitor (CHK1i) and low-dose hydroxyurea (LDHU)
- CHK1i+LDHU has minimal normal tissue toxicity
- CHK1i+LDHU treatment is equally effective in treatment-naive and treatment resistant melanomas
- CHK1i+LDHU induces immunogenic cell death and promotes pro-inflammatory cytokine expression
- CHK1i+LDHU induced anti-tumour immune response is limited by immune suppression in the tumour microenvironment



? Question

Can we and how to enhance the efficacy and duration of the anti-tumour immune response induced by CHK1i+LDHU?

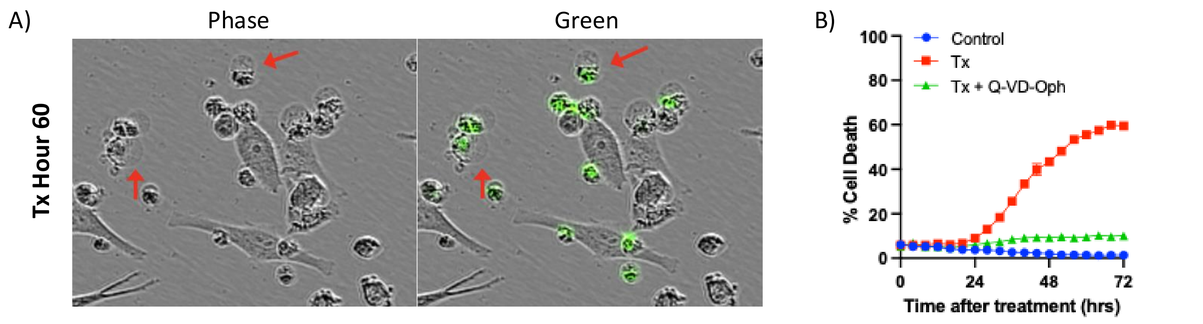
Fig 1: CHK1i+LDHU is equally effective in treatment-naive and melanomas resistant to current therapies (targeted treatments or immunotherapies) [1]



Dose response of the indicated melanoma cell lines to dabrafenib, or CHK1i (SRA737) with a constant 0.2 mM HU. Cell viability was measured by resazurin assay.

[1] Zeng Z, et al. Checkpoint kinase 1 inhibitor + low-dose hydroxyurea efficiently kills BRAF inhibitor- and immune checkpoint inhibitor-resistant melanomas. Pigment Cell Melanoma Res. 2024;37(1):45-50. doi:10.1111/pcmr.13120

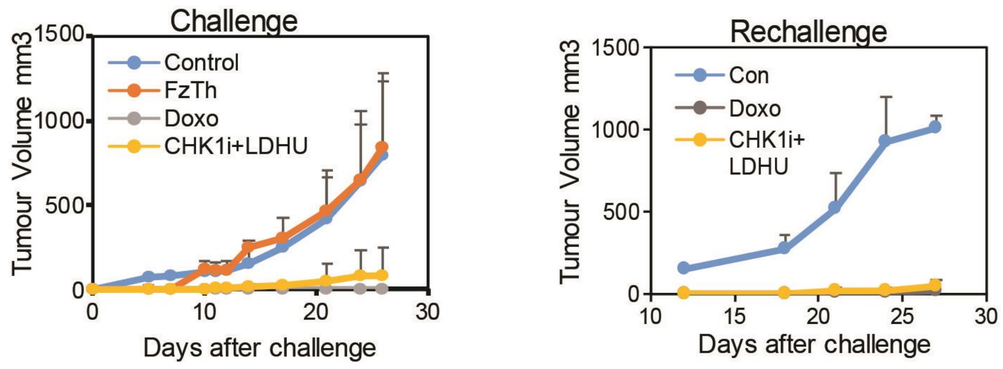
CHK1i+LDHU induces a non-canonical form of caspase-dependent pyroptosis



A) SKMEL13 and B) A2058 melanoma cells with indicated treatment and followed by IncuCyte imaging for up to three days. IncuCyte images were analysed for total cell number and percentage of dead cells (Cell Death %) measured using Sytox Green uptake.
*Tx, 1μM CHK1i (SRA737) + 0.2mM HU

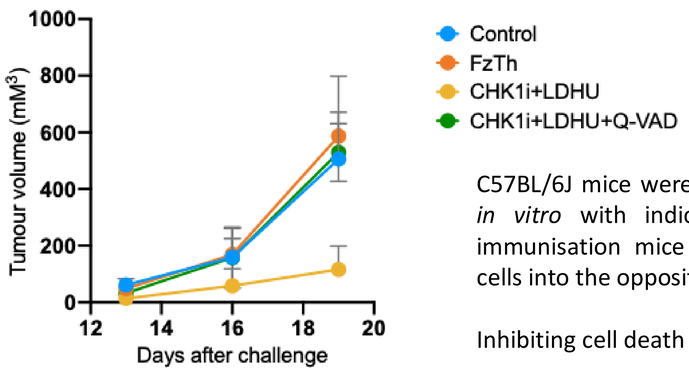
Fig 2: CHK1i+LDHU induced cell death is immunogenic

YUMMUV1.7 ICD Experiment [2]



A) C57BL/6J mice were immunised with YUMMUV1.7 cells treated *in vitro* with indicated treatments. At 10 days after immunisation mice were rechallenged with live YUMMUV1.7 cells into the opposite flank and tumour growth followed. B) 60 days after the original immunisation, tumour free mice from A) and additional control mice were challenged with live YUMMUV1.7 cells. Tumour growth was followed.

B16F10 ICD Experiment

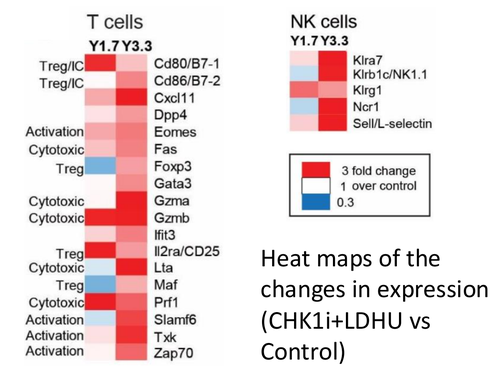


C57BL/6J mice were immunised with B16F10 cells treated *in vitro* with indicated treatments. At 10 days after immunisation mice were rechallenged with live B16F10 cells into the opposite flank and tumour growth followed.

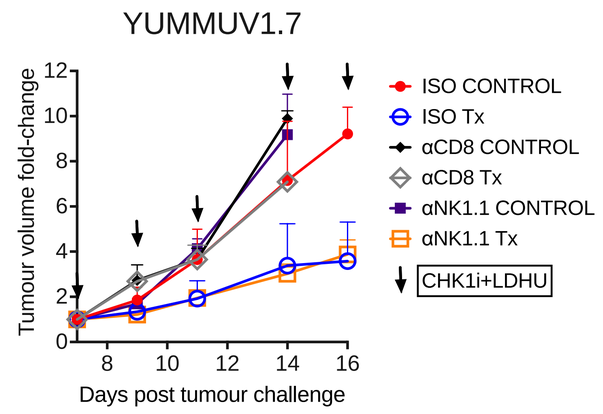
Inhibiting cell death with Q-VAD blocks immune response.

Fig 3: The adaptive immune response driven by cytotoxic CD8+ T-cells is critical for tumour control induced by CHK1i+LDHU

NanoString Immune Profiling Panel



Heat maps of the changes in expression (CHK1i+LDHU vs Control)



The responses were dependent on CD8+ T cells and involved NK cell activation.

Fig 4: Enhanced signals from the PD-1/PD-L1 pathway were observed in YUMMUV1.7 tumours with CHK1i+LDHU treatment

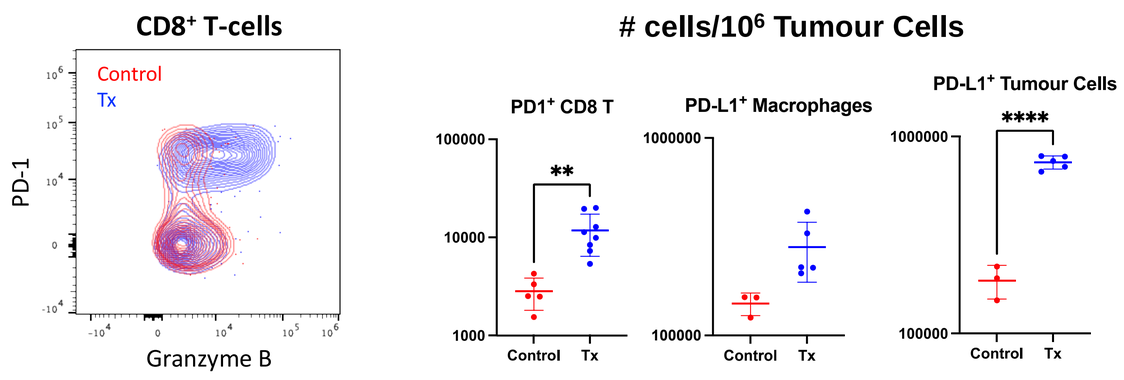
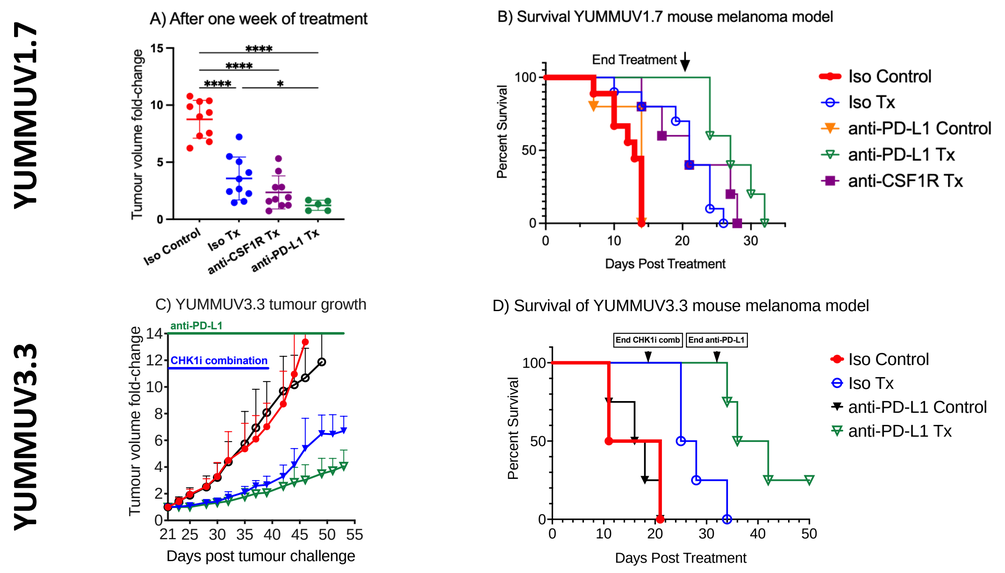


Fig 5: Anti-PD-1 had little effect on tumour response to our treatment [2], depleting CSF-1R+ macrophages and anti-PD-L1 enhanced anti-tumour response in melanomas

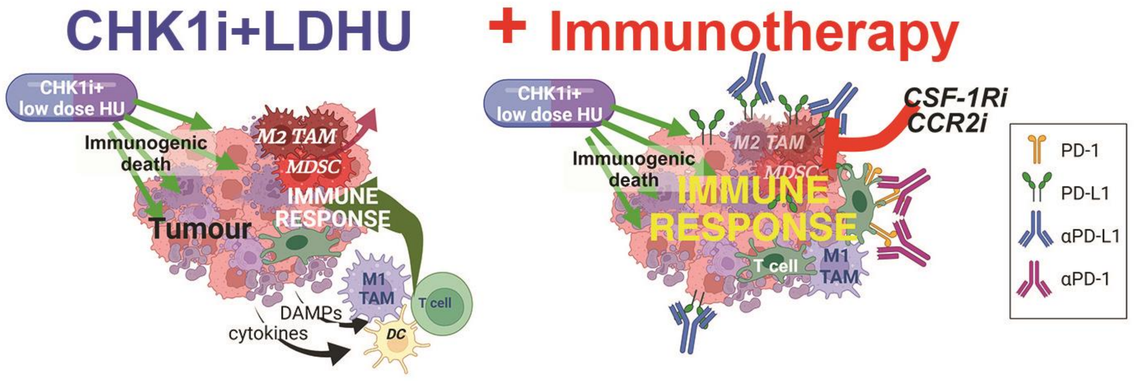


A), C) Tumour burden and B), D) Kaplan-Meier graph of tumour bearing mouse under indicated treatments

[2] Proctor M, et al. Targeting Replication Stress Using CHK1 Inhibitor Promotes Innate and NKT Cell Immune Responses and Tumour Regression. Cancers (Basel). 2021;13(15):3733. Published 2021 Jul 25. doi:10.3390/cancers13153733

Current ideas

CHK1i combination has the potential to be used along with other immunotherapies



Conclusions

- CHK1i+LDHU is selectively cytotoxic to tumour tissues
- CHK1i+LDHU efficiently kills BRAFi and immune checkpoint inhibitor resistant melanomas
- Triggers proinflammatory cytokine expression, immunogenic cell death and memory immune response
- Effects are mainly dependent on the adaptive immune response
- Modifies the anti-tumour immune microenvironment – myeloid and lymphoid compartments