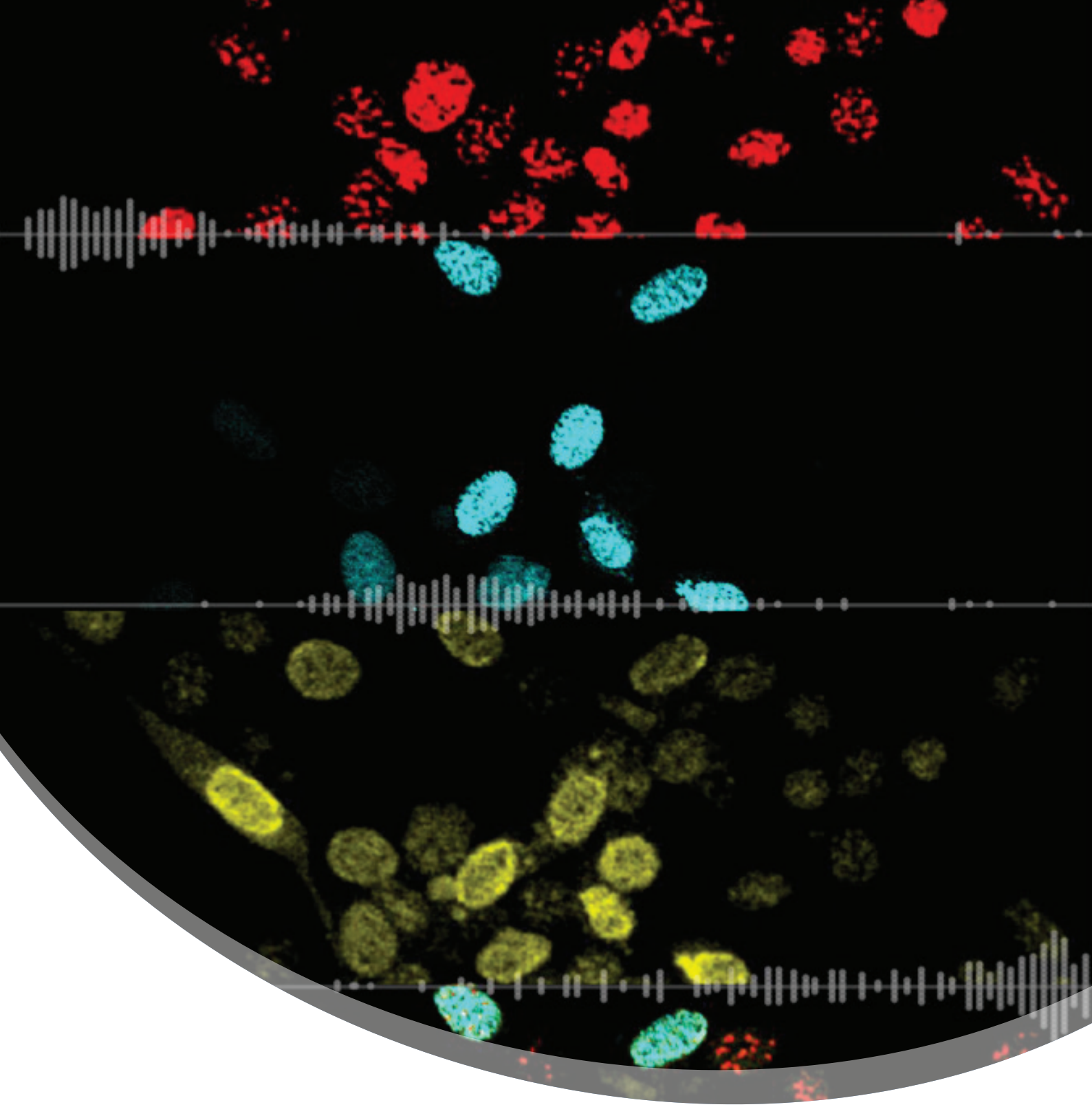


The ACRF Telomere Analysis Centre

2020 Annual Report

CHILDREN'S MEDICAL RESEARCH INSTITUTE

Jeans for Genes®

AUSTRALIAN CANCER RESEARCH FOUNDATION

Cover image:

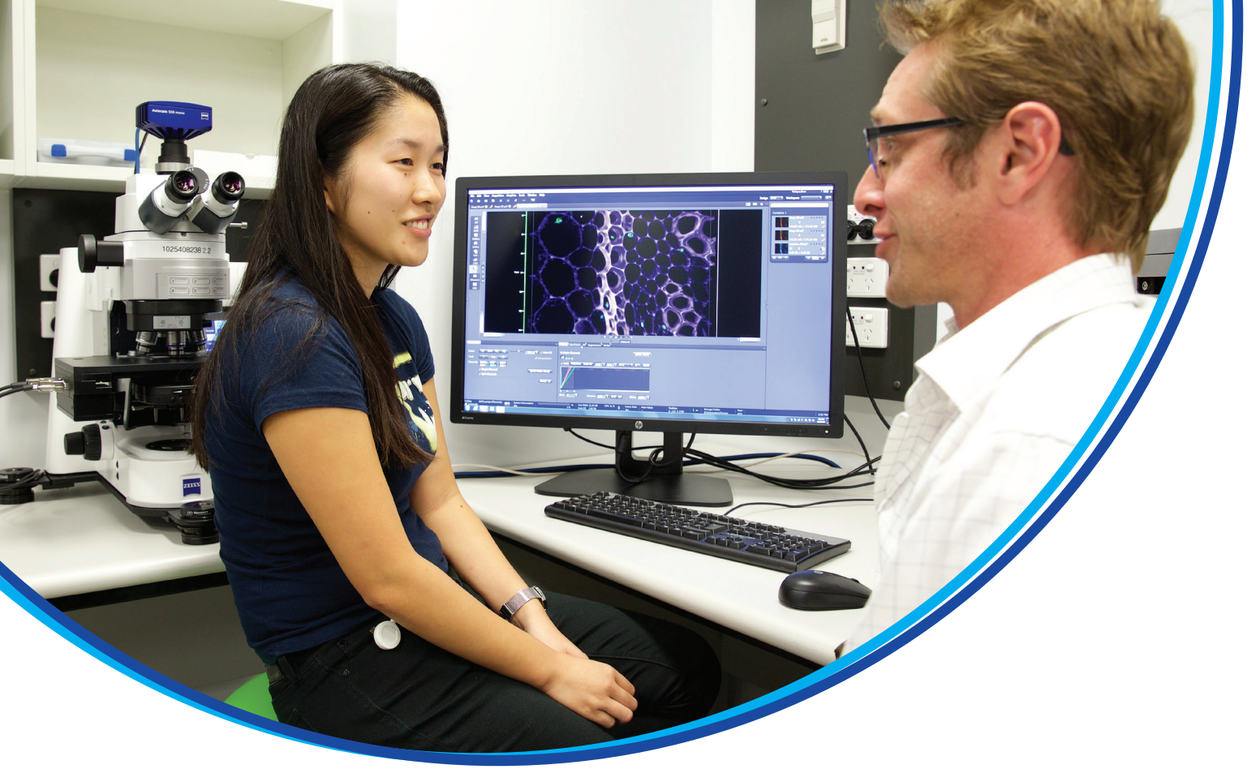
Bimolecular fluorescence complementation assay image used to quantify the heterodimers (cyan) versus homodimers (yellow) formed by TWIST1 variants in single nuclei (red). Mutations of conserved residues in the TWIST1 basic helix-loop-helix domain impaired formation of the TWIST1-TCF12 heterodimer, which is required for the differentiation of mesenchymal precursors.

Generated using equipment funded by ACRF at the Telomere Analysis Centre.

Xiaochen Fan, Ashley J. Waardenberg, Madeleine Demuth, Pierre Osteil, Jane Sun, David A.F. Loebel, Mark Graham, Patrick P.L. Tam and Nicolas Fossat (2020)
TWIST1 homodimers and heterodimers orchestrate lineage-specific differentiation.

Published in *Molecular and Cellular Biology, Vol 40 Issue 12 with the image on the cover of issue 16 of the Journal.*

Contents



Overview

The primary scientific focus of the ACRF Telomere Analysis Centre (ATAC) is the study of telomeres, the structures at chromosome ends; their roles in cell proliferation, cancer and ageing; with a secondary focus being to support a broad range of medical and biological research projects.

ATAC was the brainchild of a unique group of telomere researchers from a broad range of scientific and medical backgrounds and skill sets including clinical haematology, medical oncology, and laboratory-based research. This large consortium of telomere researchers utilise cutting-edge equipment and specialised research techniques in this internationally unique Centre for collaborative telomere research.

The Centre focuses on four key components of telomere-related research, including:

1. **Telomere Length Analysis.** ATAC supports collaborative epidemiological and clinical studies throughout Australia, in which telomere length is used as a biomarker.

2. **Automated Microscopy**. CMRI pioneers the use of automated scanning for metaphase cells in telomere research, and the ATAC equipment allows faster image analysis procedures to be developed for analysing cells at all phases of the cell cycle.

3. **High-Resolution Fluorescence Microscopy**. The length of individual telomeres, telomere structure, and telomere-interacting proteins are all critically important for telomere function and research. The high-resolution microscope technology at ATAC greatly enhances researchers' ability to study these characteristics.

4. **Live Cell Imaging**. Telomere structure and function changes dynamically during the cell cycle. Technology at ATAC places our research groups at the forefront of this field by providing researchers with the ability to study telomere dynamics in live cells.

The installation of the Centre's equipment, which was supported by the Australian Cancer Research Foundation (ACRF) and the Ian Potter Foundation was completed in 2015. On May 21, 2015 ATAC was officially opened by The Hon. Pru Goward, NSW Minister for Medical Research. Since then, scientific researchers have taken full advantage of ATAC's custom-built laboratory space on Level 2 of the Children's Medical Research Institute's tower.

Achievements

Facility Use.

2019 saw imaging equipment used at the highest levels to date. 2020 would have seen a similar increase in use; however, the change in recommended working conditions required to keep Australia safe from COVID-19 meant that fewer experiments were conducted on site and fewer experiments were carried out on the Centre's instruments.

Despite this, there were still over 19,000 hours of time booked on equipment in the Centre (this is the equivalent of 10 person-years of work). The nine main microscopes saw approximately 12,000 hours of use in the year; the remaining use was on image analysis computers managed by the Centre.

The results of these experiments carried out in the Centre will:

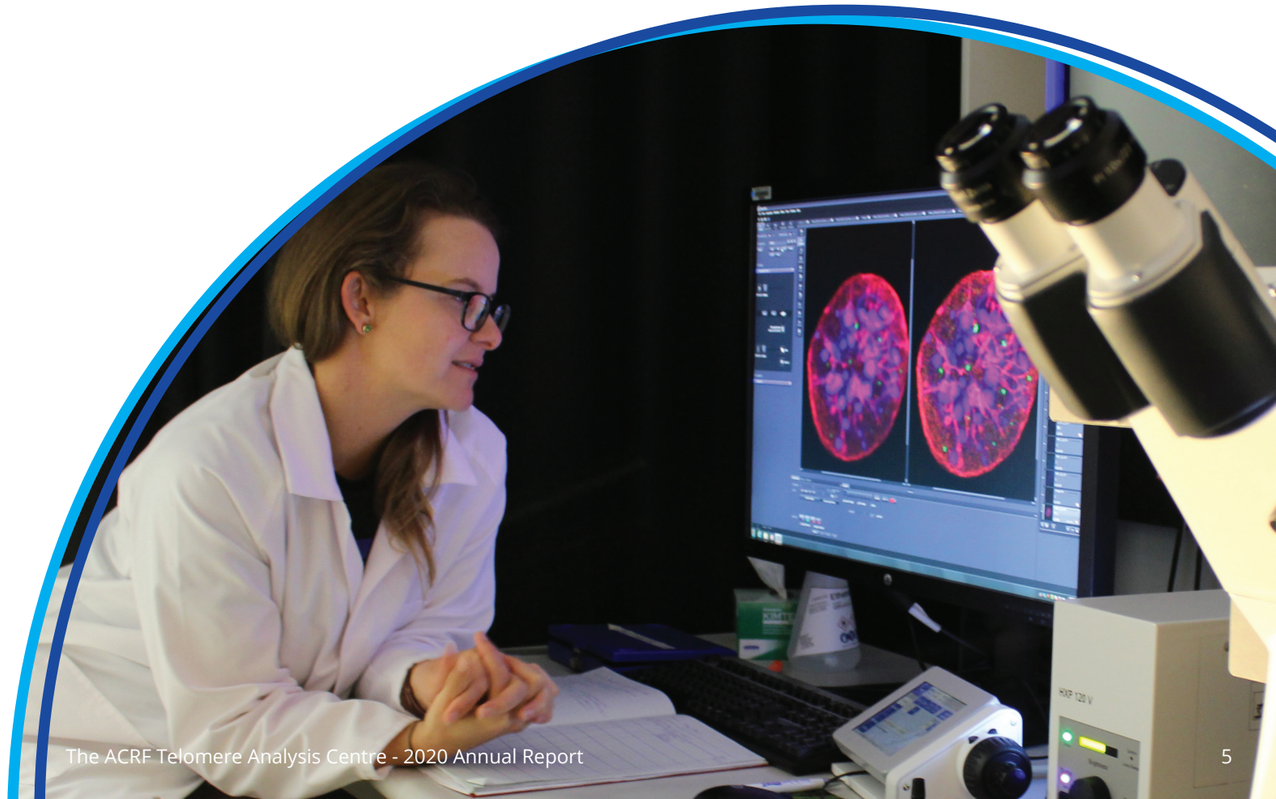
- continue the studies of research students (work-experience, Honours, M.Sc. Ph.D.);
- continue the research of many teams within the Westmead Health Precinct;
- contribute to funding proposals;
- constitute results for manuscripts;
- all with the aim of finding treatments and cures for cancer and other diseases.

Facility Publications.

In 2020, investigators who had been using the Centre over the last few years saw their research published in some of the best journals in the world. Manuscripts in the Journals "*Cell*" and "*Nature*" represent some of the most important discoveries of the year. In total, fourteen manuscripts describing work where critical discoveries were made using centre equipment were published in 2020. These include studies contributing to our understanding of cancer but also DNA, chromosomal, nuclear, and cellular biology and development.

Facility Grants.

The work of Centre researchers was also recognised in grant success. In 2020, five Australian Research Council or National Health & Medical Research Council grants were awarded to facility users, totalling over $$2,400,000 for 2021-2023.



Centre Use

	2016	2017	2018	2019	2020
Number of researchers	43	48	51	55	88
Number of research groups	10	11	13	16	20
Number of research projects	29	31	37	39	52

The number researchers utilising the Centre in 2020 was 88, a significant increase from previous years. These researchers represented 22 different research groups. The research groups include 15 from Children's Medical Research Institute and 5 based elsewhere in the Westmead Health Precinct, indicating that the facility is being accessed by a wider range of researchers. We estimate that in 2020 only around a third of the research projects do not have cancer as a principal focus.

Centre Publications

Pan X, Chen Y, Biju B, Ahmed N, Kong J, Goldenberg M, Huang J, Mohan N, Klosek S, Parsa K, Guh CY, Lu R, Pickett HA, Chu HP, Zhang D (2019). **FANCM suppresses DNA replication stress at ALT telomeres by disrupting TERRA R-loops.** ***Scientific Reports*** 9(1), 19110. https://doi.org/10.1038/s41598-019-55537-5

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Dephosphorylation of YB-1 is required for nuclear localisation during G2 phase of the cell cycle. ***Cancers*** 12(2), 315. https://doi.org/10.3390/cancers12020315

Hamada H and Tam P (2020)**. Diversity of left-right symmetry breaking strategy in animals.** ***F1000 Research*** 9, F1000 Faculty Rev-123. https://doi.org/10.12688/f1000research.21670.1

Rick C. Betori, Yue Liu, Rama K. Mishra, Scott B. Cohen, Stephen J Kron, and Karl A. Scheidt. **Targeted Covalent Inhibition of Telomerase**. ***ACS Chemical Biology*** 15(3), 706–717. https://doi.org/10.1021/acschembio.9b00945

Tomlinson, C.G.; Sasa, G.; Aubert, G.; Martin-Giacalone, B.; Plon, S.E.; Bryan, T.M.; Bertuch, A.A.; Gramatges, M.M. (2020)**. Clinical and functional characterization of telomerase variants in patients with pediatric acute myeloid leukemia/myelodysplastic syndrome.** ***Leukemia*** 35(1), 269–273. https://doi.org/10.1038/s41375-020-0835-8

Alexander P. Sobinoff and Hilda A. Pickett (2020)**. Mechanisms that drive telomere maintenance and recombination in human cancers.** ***Current Opinion in Genetics and Development*** 60, 25–30. https://doi.org/10.1016/j.gde.2020.02.006

Vivian F. S. Kahl, Joshua A. M. Allen , Christopher B. Nelson, Alexander P. Sobinoff , Michael Lee, Tatjana Kilo, Raja S. Vasireddy and Hilda A. Pickett (2020)**. Telomere length measurement by molecular combing**. ***Frontiers in Cell and Developmental Biology*** 8, 493. https://doi.org/10.3389/fcell.2020.00493

Roger Reddel, Karen L MacKenzie and Tracy M Bryan (2020)**. End Products of Telomere Research**. ***Cell Stem Cell*** 26(6), 804–805. https://doi.org/10.1016/j.stem.2020.05.006

Kafer GR and Cesare AJ (2020)**. A survey of essential genome stability genes reveals that replication stress mitigation is critical for peri- implantation embryogenesis.** ***Frontiers in Cell and Developmental Biology***. 8: 416. https://doi.org/10.3389/fcell.2020.00416

Tomáška L, Cesare AJ, Al Turki T, and Griffith JD. Twenty years of t-loops: a case study for the importance of collaboration in molecular biology. **DNA Repair.** 94, 102901, doi:10.1016/j.dnarep.2020.102901

Xiaochen Fan, Ashley J. Waardenberg, Madeleine Demuth, Pierre Osteil, Jane Sun, David A.F. Loebel, Mark Graham, Patrick P.L. Tam and Nicolas Fossat (2020)**. TWIST1 homodimers and heterodimers orchestrate lineage-specific differentiation**. ***Molecular & Cellular Biology*** 40(11), e00663-19. https://doi.org/10.1128/MCB.00663-19

Phil Ruis, David Van Ly, Valerie Borel, Georgia R. Kafer, Afshan McCarthy, Steven Howell, Robert Blassberg, Ambrosius P. Snijders, James Briscoe, Kathy K. Niakan, Paulina Marzec, Anthony J. Cesare* and Simon J. Boulton* (2020). **TRF2-independent chromosome end protection during pluripotency.** ***Nature,*** 589(7840), 103–109. https://doi.org/10.1038/s41586-020-2960-y

Noa Lamm, Mark N. Read, Max Nobis, David Van Ly, Scott G. Page, V. Pragathi Masamsetti, Paul Timpson, Maté Biro and Anthony J. Cesare (2020). **Nuclear F-actin counteracts nuclear deformation and promotes fork repair during replication stress**. ***Nature Cell Biology*** 22(12), 1460–1470. https://doi.org/10.1038/s41556-020-00605-6

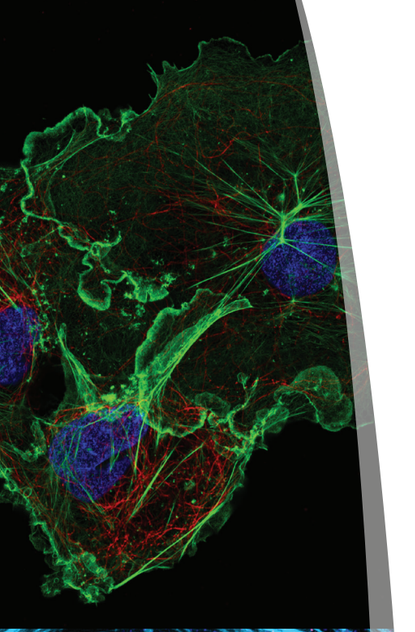
Alexandra M Pinzaru, Mike Kareh, Noa Lamm, Eros Lazzerini-Denchi, Anthony J Cesare, and Agnel Sfeir (2020)**. Replication stress conferred by POT1 dysfunction promotes telomere relocalization to the nuclear pore.** ***Genes & Development*** 34(23-24), 1619–1636. https://doi.org/10.1101/gad.337287.120

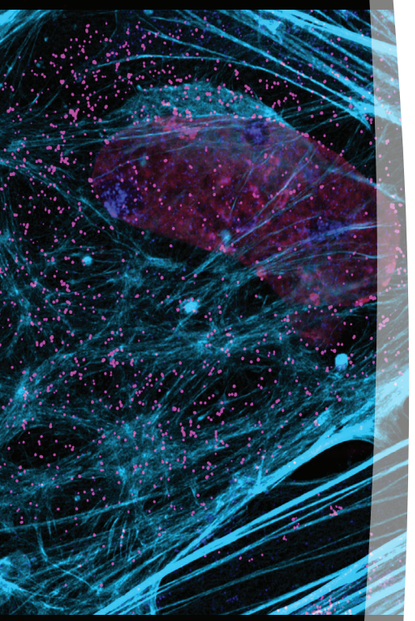
Sunali Mehta, Michael Algie, Tariq Al-Jabry, Cushla McKinney, Srinivasaraghavan Kannan, Chandra S Verma, Weini Ma, Jessie Zhang, Tara K. Bartolec, V. Pragathi Masamsetti, Kim Parker, Luke Henderson, Maree L Gould, Puja Bhatia, Rhodri Harfoot, Megan Chircop, Torsten Klemann, Scott B Cohen, Adele G Woolley*, Anthony J Cesare* and Antony Braithwaite*. (2020) **Critical role for cold shock protein YB-1 in cytokinesis.** ***Cancers*** 12(9), 2473. https://doi.org/10.3390/cancers12092473

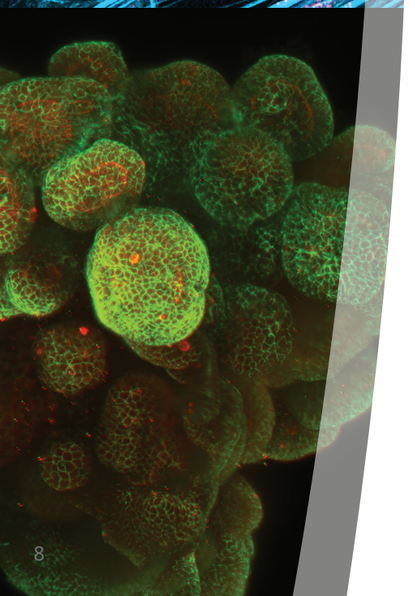
Ginn SL, Amaya AK, Liao S, Zhu E, Cunningham SC, Lee M, Hallwirth CV, Logan GJ, Tay SS, Cesare AJ, Pickett HA, Grompe M, Dilworth K, Lisowski L and Alexander IE (2020). **Efficient in vivo editing of OTC-deficient patient-derived primary human hepatocytes.** ***JHEP Reports.*** 2: 100065, doi:10.1016/j.jhepr.2019.100065

Giles KA, Gould CH, Achinger-Kawecka J, Page SG, Kafer G, Rogers S, Luu PL, Cesare AJ, Clark SJ and Taberlay PC (2021). **BRG1 knockdown inhibits proliferation through multiple cellular pathways in prostate cancer.** ***Clinical Epigenetics.*** 13:37, DOI: 10.1186/s13148-021-01023-7

Xiao L, Somers K, Murray J, Pandher R, Karsa M, Ronca E, Bongers A, Terry R, Ehteda A, Gamble LD, Issaeva N, Leonova KI, O'Connor A, Mayoh C, Venkat P, Quek H, Brand J, Kusuma FK, Pettitt JA, Mosmann E, Kearns A, Eden G, Alfred S, Allan S, Zhai L, Kamili A, Gifford AJ, Carter DR, Henderson MJ, Fletcher JI, Marshall G, Johnstone RW, Cesare AJ, Ziegler DS, Gudkov AV, Gurova KV, Norris MD and Haber M. **Dual targeting of chromatin stability by the curaxin CBL0137 and histone deacetylase inhibitor Panobinostat shows significant preclinical efficacy in neuroblastoma.** ***Clinical Cancer Research.*** In press







Centre Grants

A/Prof Anthony Cesare

NHMRC

Understanding the molecular mechanisms of cell death in radiotherapy

2021-2023; $643,856

ARC

Understanding telomere privilege in pluripotent stem cells

2021-2023; $555,892

Neil and Norma Hill Foundation

$100,000

Prof. Hilda Pickett

NHMRC

Molecular characterisation of the DBHS proteins in telomerase assembly

2021-2023; $686,246

Prof Robyn Jamieson

ORIA

A new inflammatory pathway for modulation in the retinal dystrophies

2021; $50,000

Dr. Noa Lamm-Shalem

NHMRC

Ataxia-Telangiectasia: An emerging role for inflammation in driving neurodegeneration and premature ageing

2021-2022; $82,245

Kids Cancer Alliance Project Grant

$200,000

Governance

Scientific Advisory Committee

Prof. Tracy Bryan, Head, Cell Biology Unit, CMRI

Assoc. Prof. Tony Cesare, Head, Genome Integrity Unit, CMRI

Dr. Julie Curtin, Senior Staff Specialist, Department of Haematology, Sydney Children's Hospital Network Westmead

Dr. Loretta Lau, Senior Lecturer (Conjoint), University of New South Wales

Associate Professor Karen Mackenzie, Project Manager, Cancer Research Unit, CMRI; Senior Lecturer (Conjoint), University of New South Wales

Prof. Hilda Pickett, Head, Telomere Length Regulation Unit, CMRI

Prof. Roger Reddel AO, Lorimer Dods Professor and Director; Head, Cancer Research Unit, CMRI

Management

Dr. Scott L Page, Manager, Advanced Microscopy and ACRF Telomere Analysis Centre, CMRI

Mr. Joshua Studdert, Staff Scientist, Equipment & Imaging Support, CMRI (0.5 FTE ATAC/Advanced Microscopy Centre management assistance)

Future

The Centre has continued to grow and evolve and is likely to continue to do so. There are more projects than ever, more research groups and scientists accessing the Centre, more research success measured by publications and grants, and indeed, a broader range of studies being performed. As researchers' imaging requirements become more specialised, only dedicated and well-funded centres such as this can provide the access and expertise required.

As such, the Centre now forms one of the Core Facilities promoted and supported by the Westmead Research Hub, a partnership between CMRI, The Westmead Institute for Medical Research, The Western Sydney Local Health District, CSIRO, Kids Research and the Sydney Children's Hospitals Network, The University of Sydney, and NSW Government Health Pathology. With this even greater visibility, we will be able to the support a larger and more diverse range of research needs across the Precinct and greater Sydney area.

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