Name	DINIS CALADO
Position	Group Leader (2nd 6)
Year joined *(Crick or founder institute)*	2013

Career History

2000-2006: Graduate Student, Gulbenkian Institute, Gulbenkian Foundation,Lisbon, Portugal
2006-2010: Postdoctoral Fellow at PCMM-IDI Children's Hospital, Harvard Medical School, USA (2010)
2010-2013: Special Fellow of the Leukemia Lymphoma Society PCMM-IDI Children's Hospital, Harvard Medical School, USA / Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin, Germany

Major Awards, Honours and Prizes

2000-2004: Graduate Fellowship, Foundation for Science and Technology, Portugal
2005-2009: Post-Graduate Fellowship, Foundation for Science and Technology, Portugal
2010-2013: Career Development Award, Special Fellow of the Leukemia Lymphoma Society, USA
2011: Waterman Family Leukemia Lymphoma Society Researcher, US
2013-2019: Career Development Award, Medical Research Council, UK

Membership of external committees, editorial boards, review panels, SABs etc

2018-present: Bloodwise Expert Panel.

Lab Name	***Immunity and Cancer Laboratory***

Research programme and achievements

The Immunity and Cancer Laboratory studies the germinal center (GC) B-cell reaction, a stage of adaptive immunity critical for long-term protection from infection and underlying vaccination success. The evolutionary trade-off of such a reaction is the occurrence of autoimmunity and haematological cancers.

GCs are formed following the recognition of an infectious or vaccine antigen by the B-cell receptor (BCR) of a mature B-cell. This process gives rise to B-cells carrying BCRs with a wide range of affinities for antigen. The currently favoured model proposes that only those B-cells carrying BCRs with higher antigen affinity are selected to survive.

Once selected, GC B-cells can differentiate into plasma cells (PC) in which the BCR is converted into a secreted form, the antibody, that binds antigen and aids the elimination of the infectious agent. In a far more obscure process, GC B-cells can also form memory B-cells (MBC). These “experienced” B-cells are essential upon re-infection, or exposure to the vaccine antigen.

Clearly, selection of B-cells is critical for GC function, but strict affinity-dependent selection does not explain the retention of a wide range of antigen affinities in the reaction, which may be required for broadly neutralising antibody formation as seen in HIV and Influenza patients. It is also unclear whether MBCs are derived from selected cells. Research in this area is hampered by the exceedingly low number of GC B-cells undergoing selection and the absolute requirement of *in vivo* studies.

Previously, we and others identified the expression of the cell cycle regulator MYC as a marker of GC B-cells undergoing selection. Using this marker, we have characterised GC B-cell selection in unprecedented depth. Single-cell RNA-sequencing revealed unexpected diversity of selected GC B-cells and allowed the identification of surface markers defining sequential clusters of selected cells by flow-cytometry. These studies demonstrated that GC selection is in fact highly permissive and that selected B-cells include those with lower antigen affinity. We also identified clusters of selected B-cells that are candidate PC and MBC precursors. In related work, we showed that the MBC fate within selected B-cells is restricted through the action of a transcriptional repressor complex formed by MYC and MIZ1 (ZBTB17). These works enhance our understanding of GC B-cell selection, and pave the way for interventions that tailor the GC B-cell response to meet specific requirements for infection control and prevention.

In depth analysis of selected GC B-cells may also aid the identification of lymphoma precursors. Activated B-Cell Diffuse Large Cell Lymphomas (ABC-DLBCL) display enforced NF-κB activation downstream of genetic mutations. NF-κB activation plays a crucial role in B-cell to PC differentiation and the gene is transiently expressed, together with MYC, in PC precursors. MYC expression is then suppressed as PC differentiation ensues, allowing cells to enter a post-mitotic state.

ABC-DLBCLs display the phenotype of a B-cell blocked during PC differentiation, so we wondered whether MYC overexpression could be in part responsible for this. We found instead that NF-κB synergised with MYC to produce a cancer with a PC-like phenotype, demonstrating that MYC overexpression does not interfere with B-cell phenotype loss. The cancer cells had a plasmablast phenotype (i.e. of a PC at an early differentiation stage), and NF-κB and MYC co-activation specifically made these cells addicted to IL-6.

These findings are relevant for cancer therapy, as MYC overexpression is linked to treatment resistance and relapse. We collaborated with Ed Tate’s laboratory at Imperial College to show that inhibition of myristoylation was synthetically lethal with high levels of MYC in cancer cells, including in the extremely hard-to-treat double-hit lymphomas that carry concurrent MYC and BCL2 translocations. These studies led to a patent approval, the set-up of the company “Myricx” and to our laboratories being awarded the “2019 Sir David Cooksey Prize in Translation”.

Research outputs

Barbosa RR, Xu AQ, D'Andrea D, Copley F, Patel H, Chakravarty P, Clear A, Calaminici M, Janz M, Zhang B, Schmidt-Supprian M, Wang J, Gribben JG, Tooze R, Fitzgibbon J, Franzoso G, Rajewsky K, Calado DP. (2020) *Co-activation of NF-κB and MYC renders cancer cells addicted to IL6 for survival and phenotypic stability.* bioRxiv 2020.04.12.038414. DOI: 10.1101/2020.04.12.038414.
NF-κB and MYC are found co-deregulated in human B and plasma-cell cancers. Using a mouse system to trace cell lineage and oncogene activation, we found that NF-κB/MYC co-deregulation produced cancers with a plasmablast-like phenotype similar to human plasmablastic lymphoma and also t(8;14)[MYC-IGH] multiple myeloma. Notably, in contrast to NF-κB or MYC activation alone, co-deregulation rendered cells addicted to IL6 for survival and phenotypic stability. We propose that conflicting oncogene-driven differentiation pressures can be accommodated at a cost in poorly-differentiated cancers.

Xu AQ, Barbosa RR, Calado DP. (2020) *Genetic timestamping of plasma cells in vivo reveals tissue-specific homeostatic population turnover*. eLife 2020,9:e59850. DOI: 10.7554/eLife.59850.
Plasma cells (PCs) are essential for protection from infection, and at the origin of incurable cancers. Current studies do not circumvent the limitations of removing PCs from their microenvironment and confound formation and maintenance. Here we characterize a genetic tool in the mouse that permits first-ever specific genetic manipulation in PCs in vivo, across immunoglobulin isotypes. This tool paves the way for an in-depth mechanistic understanding of PC biology and pathology in vivo, in their microenvironment.

Toboso-Navasa A, Gunawan A, Morlino G, Nakagawa R, Taddei A, Damry D, Patel Y, Chakravarty P, Janz M, Kassiotis G, Brink R, Eilers M, Calado DP. (2020) *Restriction of memory B cell differentiation at the germinal center B cell positive selection stage.* J Exp Med 217(7):e20191933. DOI: 10.1084/jem.20191933.
Memory B cells (MBCs) are key for protection from pathogen reinfection. However, it is mechanistically unclear how MBCs differentiate. We found that the complex formed by the transcription factors MYC and MIZ1 [ZBTB17] represses the expression of genes associated with MBC differentiation and that mice lacking MYC-MIZ1 complexes increased MBC differentiation. Thus, MYC-MIZ1 complexes restrict MBC differentiation. We propose that interventions that modulate the activity of MYC–MIZ1 complexes may tailor the immune response to meet individual requirements for infection control and prevention.

Kallemeijn WW, Lueg GA, Faronato M, Hadavizadeh K, Goya Grocin A, Song OR, Howell M, Calado DP, Tate EW. (2019) *Validation and invalidation of chemical probes for the human N-myristoyltransferases*. Cell Chem Biol. 26(6):892-900.e4. DOI: 10.1016/j.chembiol.2019.03.006.
On-target, cell-active chemical probes are of fundamental importance in chemical and cell biology, whereas poorly characterized probes often lead to invalid conclusions. Human N-myristoyltransferase (NMT) has attracted increasing interest as target in cancer and infectious diseases. Here we report an in-depth comparison of five compounds widely applied as human NMT inhibitors, using a combination of quantitative whole-proteome N-myristoylation profiling, biochemical enzyme assays, cytotoxicity, in-cell protein synthesis, and cell-cycle assays. We find that N-myristoylation is unaffected by 2-hydroxymyristic acid (100 μM), D-NMAPPD (30 μM), or Tris-DBA palladium (10 μM), with the latter compounds causing cytotoxicity through mechanisms unrelated to NMT. In contrast, drug-like inhibitors IMP-366 (DDD85646) and IMP-1088 delivered complete and specific inhibition of N-myristoylation in a range of cell lines at 1 μM and 100 nM, respectively. This study enables

the selection of appropriate on-target probes for future studies and suggests the need for reassessment of previous studies that used off-target compounds.

Nakagawa R, Toboso-Navasa A, Schips M, Young G, Bhaw-Rosun L, Llorian-Sopena M, Chakravarty P, Sesay AK, Kassiotis G, Meyer-Hermann M, Calado DP. (2021) ***Permissive selection followed by affinity-based proliferation of GC light zone B cells dictates cell fate and ensures clonal breadth*****. Proc Natl Acad Sci USA 118(2):e2016425118. DOI: 10.1073/pnas.2016425118.**
Memory B cells (MBCs) and plasma cells (PCs) are formed during the so-called germinal center (GC) B cell reaction. In the GC reaction B cells mutate their B cell receptor (BCR) genes and those that acquire a higher-affinity BCR for a pathogen antigen are presumably selected to survive and differentiate, whereas B cells carrying a lower-affinity BCR die. However, this cannot explain retention of GC B cells with varied BCR affinities and the formation of MBCs that normally carry lower-affinity BCRs. This work re-defines selection of GC B cells as permissive to ensure clonal diversity and broad protection.