Potentiation of Anti-Myeloma Activity of Daratumumab With Combination of Cyclophosphamide, Lenalidomide or Bortezomib via a Tumour Secretory Response That Greatly Augments Macrophage-Induced ADCP

Athina Rigalou, MSc[1]; Aideen Ryan, PhD[1]; Alessandro Natoni, PhD[2]; Christopher Chiu, PhD[3]; A. Kate Sasser, PhD[4]; Michael E. O'Dwyer, MD[5,6]

[1]Biosciences, National University of Ireland, Galway, Galway, Ireland; [2]Department of Haematology, National University of Ireland, Biosciences, Galway, Ireland; [3]Oncology Heme Translational Research Group, Janssen Research & Development, LLC, Spring House, PA, USA; [4]Janssen Research & Development, LLC, Spring House, PA, USA; [5]Department of Haematology, National University of Ireland, Biosciences, Galway, Ireland; [6]Department of Haematology, University Hospital Galway, Galway, Ireland.

Introduction

Daratumumab (DARA) is the first CD38-directed monoclonal antibody (moAb) approved anywhere in the world as well as the first moAb approved for the treatment of multiple myeloma (MM). While currently approved as monotherapy, recent results from randomized trials have shown dramatic activity in relapsed MM patients in combination with both lenalidomide (Len) and bortezomib (Bor) and DARA is likely to become an intrinsic component of MM regimens in the future (Rajkumar and Kyle NEJM, 2016). Based on pre-clinical data, DARA is thought to have pleiotropic mechanisms of activity, killing myeloma cells via a direct anti-CD38 effect, induction of complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Therefore, we focused our attention on potential mechanisms of enhancing macrophage (Mϕ)-mediated ADCP. Previously, Pallasch reported that resistance to Mϕ-mediated killing in lymphoid malignancy can be overcome by combination regimens involving therapeutic antibodies and chemotherapy (Pallasch et al Cell, 2014). Specifically, cyclophosphamide (Cy) was shown to induce an acute secretory activating phenotype from treated tumour cells, which led to Mϕ infiltration and phagocytic activity in the bone marrow. Given this background, we sought to establish the existence of this phenomenon in MM in the context of drugs likely to be used in combination with DARA, i.e. Cy, Len and Bor, respectively.

Hypothesis

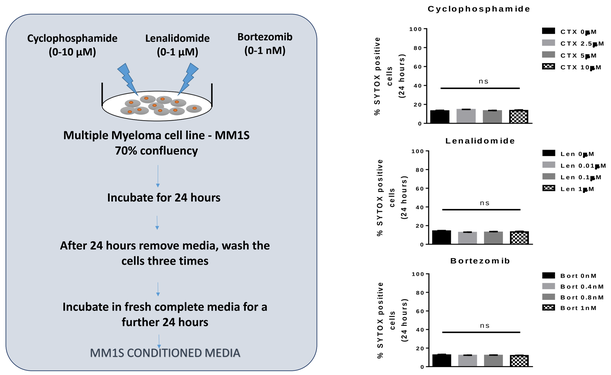
Low dose chemotherapy will enhance a secretory phenotype in MM cells that will influence macrophage migration and activation that can be harnessed to potentiate ADCP of daratumumab targeted MM cells

Materials and Methods

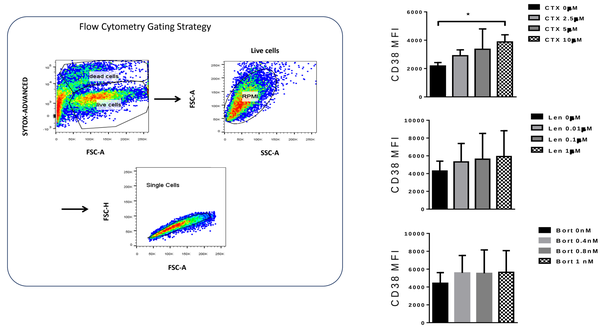
MM1S MM cells were conditioned with low dose single agents, Cy, Len and Bor for 24 hours. Media containing single agent chemotherapy was removed and replaced with fresh media for 24 hours (TCM). THP-1 Mϕ were conditioned with TCM for up to 48 hours and then incubated at 2:1 effector target ratio with CFSE labelled MM1S cells for 18 hours in the presence or absence of DARA or isotype control antibody. The % of DARA-specific tumour cell clearance was calculated as the number of live CFSE labelled tumour cells in DARA-treated cells/Isotype-treated cells. Additionally, Mϕ were pre-incubated with cytochalasin D (CytoD), an inhibitor of actin polymerization, to determine if the mechanism of tumour cell clearance is mediated by Mϕ mediated ADCP. MM1S expression of CD47, as well as Mϕ expression of Fc receptor expression, CD32 and CD64 was analysed by flow cytometry. Mϕ migration was assessed using transwell assays and determined by enumerating the absolute number of fluorescently labelled Mϕ that migrated to control, Cy, Len and /or Bor TCM.

Results

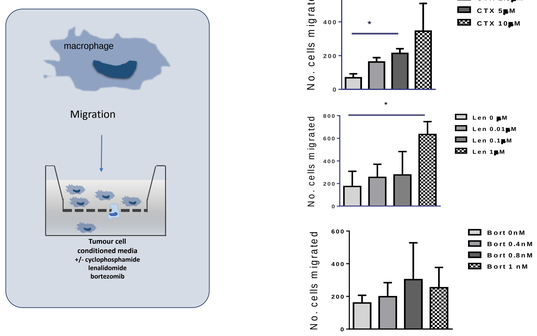
Low dose cyclophosphamide, lenalidomide or bortezomib does not induce cell death in MM cells



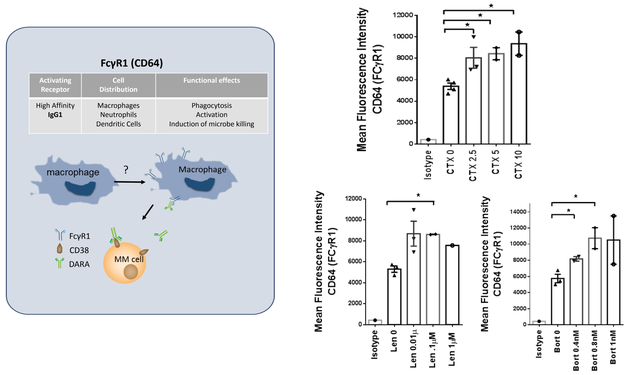
CD38 is upregulated on MM cells 24 hours following cyclophosphamide, lenalidomide or bortezomib treatment



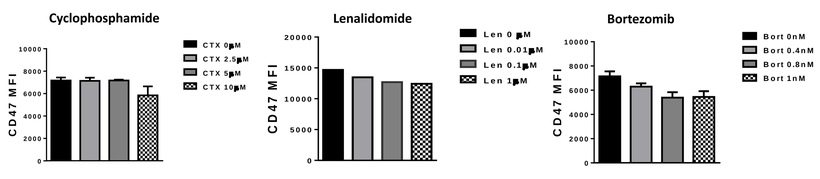
Macrophage migration is enhanced towards tumour cell media of cyclophosphamide, lenalidomide, bortezomib treated MM cells



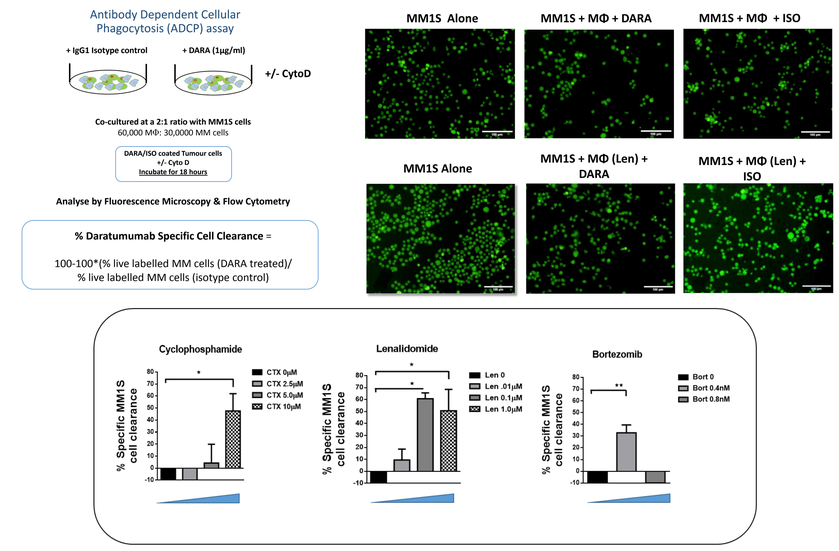
Macrophage FcγRI expression is induced via a tumour secretory response following treatment of MM cells with low dose chemotherapy



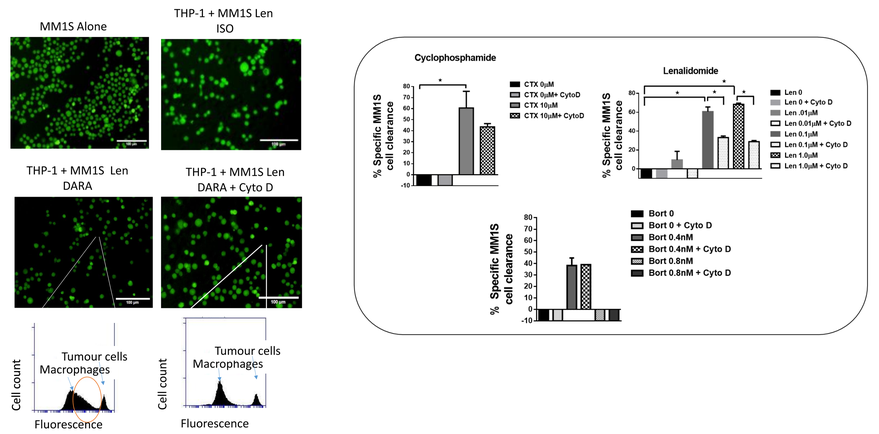
MM1S cell surface expression of CD47 is reduced 24 hours following cyclophosphamide and bortezomib treatment



Potentiation of anti-myeloma activity of daratumumab with combination of cyclophosphamide, lenalidomide or bortezomib

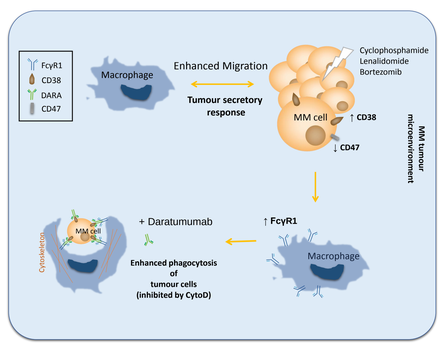


Exposure of Mϕ to TCM from Cy, Len and Bor treated MM1S cells significantly enhanced % DARA specific clearance of MM1S cells ($p<0.01$), when compared to control untreated TCM



This effect was partially reversed by incubation of Mϕ co-cultures with CytoD ($p<0.01$), indicating ADCP as one of the likely mechanisms of tumour cell clearance.

Summary



Exposure of MM cells to both low doses of Cy, Len and Bor leads to a secretory response, which, along with downregulation of CD47, greatly augments Mϕ induced ADCP of DARA coated MM cells. This effect may explain in part the dramatic synergy observed between Len, Bor and DARA in clinical trials and provides a rationale for combining Cy with DARA in the clinic for this anti-tumour effect.