

[Abstract 07]

CONSTITUTIVE EXPRESSION OF CD40L ON BONE MARROW MAST CELLS SUPPORTS THE GROWTH OF LYMPHOPLASMACYTIC CELLS IN PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA.

Olivier Tournilhac^{1,4*}, Daniel Ditzel Santos^{1,4*}, Lian Xu^{1*}, Jeffery Kutok^{3,4*}, Yu Tsu Tai^{2,4*}, Steven Le Gouill^{2,4*}, Evdoxia Hatjiharissi^{1,4*}, Laurie Catley^{2,4*}, Zachary Hunter^{1*}, Andrew Branagan^{1*}, Kenneth C Anderson^{2,4} and Steven P Treon^{1,4}. ¹ Bing Program for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute; ² Jerome Lipper Multiple Myeloma Center, Dana Farber Cancer Institute; ³ Brigham And Women's Hospital and ⁴ Harvard Medical School, Boston, MA, 02115, USA.

CD40 ligand (CD40L) is a potent inducer of normal and malignant B-cell proliferation through interaction with CD40. We and others have observed excess mast cells (MC) in bone marrow (BM) biopsies of WM patients, which are commonly found admixed with tumor aggregates. (Tournilhac et al, JCO 2004, 22:571S). We therefore sought to clarify the role of MC in WM. Co-culture of 0.5% paraformaldehyde fixed, or sublethally irradiated HMC-1, LAD, and KU mast or basophilic cell lines and sorted BM lymphoplasmacytic cells (LPC) from 10 WM patients resulted in MC dose-dependent tumor colony formation and/or proliferation as assessed by ³H-thymidine uptake studies. As demonstrated by immunohistochemical, multicolor flow cytometric (CD117⁺FcεRI⁺) and/or RT-PCR analysis, CD40L was expressed on BM MC from 29 of 31 (94%), 11 of 13 (85%), and 7 of 9 (78%) of WM patients, respectively. In contrast, cell surface CD40L expression was not detected by immunohistochemistry (p=0.00005) and flow cytometry (p=0.003) in 5 normal donors, and only faint expression for 1 of 5 normal donors by RT-PCR (p=0.09). Moreover, by multicolor flow cytometry, CD40 was expressed on BM tumor cells from 14/17 (83%) patients. CD40 functionality was confirmed either by the G28.5 CD40 agonistic antibody which induced dose dependent proliferation or by the rh-CD40L which partly prevented serum starvation-induced-apoptosis of WM LPC from 4/4 and 3/4 patients respectively. Importantly, expansion of tumor cells from 3 of 4 patients in mixed cultures with paraformaldehyde fixed MC was blocked in a dose dependent manner by use of a CD40L blocking protein (CD40:Fc). These studies demonstrate that CD40L is constitutively expressed on the cell surface of BM MC in WM and support the growth of WM tumor cells, and therefore provide the framework for therapeutic targeting of MC and MC-WM cell interactions in WM.