P1303 POTENTIATING ANTI-CD20 MONOCLONAL ANTIBODY THERAPY BY TARGETING COMPLEMENT C3 ACTIVATION FRAGMENTS COVALENTLY DEPOSITED ON LYMPHOMA CELLS

Topic: 20. Lymphoma Biology & Translational Research

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Background: The addition of monoclonal antibodies (mAbs) to chemotherapy or targeted agents improves survival of lymphoma patients. Fc receptor-dependent effector mechanisms achieve tumor cell killing but also can induce antigen loss through trogocytosis, which is mediated by Fc receptors on acceptor cells, contributing to treatment failure. Initiation of the complement cascade by cell-bound mAbs also deposits complement C3 activation fragments on the cell surface and this can promote downstream lysis of target cells by the membrane attack complex of complement. However, in the case of chronic lymphocytic leukemia (CLL) it is well established that not all of the mAb-targeted B cells are killed during these reactions. Instead, substantial numbers of surviving B cells that have lost CD20 due to trogocytosis and are covalently tagged with the complement breakdown product C3d are readily demonstrable in blood and bone marrow. Antigen loss due to trogocytosis with several types of mAbs has been demonstrated in lymphoma as well as in multiple myeloma.

Aims: We sought to test the hypothesis that covalently attached C3d fragments deposited on B cells by anti-CD20 mAbs constitute a neoantigen suitable for targeting antigen loss variants and, when used in combination with a complement fixing mAb, can increase efficacy of mAb therapy.

Methods: We generated mouse and rabbit mAbs specific for C3d and selected three mAbs that bind distinct epitopes to construct human IgG1 chimeric mAbs for further study. We obtained primary CLL cells from patients before and during treatment with ofatumumab on a clinical study conducted at the NIH (NCT01145209) and used lymphoma cell lines HBL2 (Mantle Cell Lymphoma) and SUDHL6 (Diffuse Large B cell Lymphoma) to test in vitro and in vivo efficacy of anti-C3d antibodies. Lymphoma cell lines were injected subcutaneously, mAbs were administered i.p. starting one week after tumor challenge and continued for 4-8 weekly injections and mice were sacrificed when predetermined limits of tumor burden were reached.

Results: For initial proof-of-concept, we demonstrated binding of a murine anti-C3d mAb (C8) to CLL cells from patients obtained 24 hours after ofatumumab administration; these cells had lost CD20 (negative on Western blot) and carried covalently-bound C3d. C8 selectively bound to C3d-opsonized CLL cells at low nanomolar concentrations and effectively killed these cells in a patient-derived xenograft mouse model. However, C8 did not react with murine C3d. Thus, for further in vivo studies, we used rabbit human IgG1 chimeric mAbs that cross-react with murine C3d. All 3 mAbs were able to mediate phagocytosis by monocyte derived human macrophages. In lymphoma xenografts, co-administration of a rabbit anti-C3d mAb with either rituximab or ofatumumab was more effective than the anti-CD20 mAb alone: in HBL xenografts median survival increased from 35 days with ofatumumab alone to 63 days for the combination (P=0.012). In SUDHL6 xenografts, median survival was 114 days for anti-CD20 (either rituximab or ofatumumab) treated mice and not reached for mice treated with anti-CD20 combined with an anti-C3d mAb (P=0.008), and tumor-free survival at 6 months was 20% for mice treated with an anti-CD20 mAb alone and 75% for mice treated with the combination. In surviving mice, there was no evidence of any adverse side effects or tissue damage.

Summary/Conclusion:

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Our findings indicate that C3d-targeting with a specific mAb can provide a decisive “second hit” to enhance the efficacy of complement fixing mAbs commonly used in lymphoma therapy.