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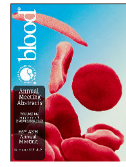

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Evidence of Defective Cell-Autonomous Signalling By B Cell Receptors in CLL Stereotyped Subset #1

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B cell receptor (BCR)-mediated signalling is central in the pathogenesis of chronic lymphocytic leukaemia (CLL), and represents a target for pharmacological intervention. Notably, so far characterized CLL-derived BCRs are capable of triggering cell-autonomous intracellular signalling through homotypic interactions without requirement for exogenous antigens.

Approximately 41% of all CLL cases can be classified in subsets based on homologous, otherwise known as stereotyped, BCRs expressed by the malignant clones, sharing remarkably similar sequences in the VH CDR3 region and highly restricted immunoglobulin heavy and/or light variable genes. Since cases within a subset display clinicobiological consistency in contrast with the pronounced heterogeneity of CLL at large, analysis of the mechanism of BCR-mediated signalling can unveil correlations between molecular details of BCR activation and the course of the disease. Our present study focused on BCRs of stereotyped subset #1, the second largest stereotyped subset, known for a particularly aggressive clinical course. Cases belonging to subset #1 express unmutated IGHV genes of phylogenetic clan I (namely, IGHV1, IGHV5 and IGHV7 subgroup genes) partnered by light chains encoded by the IGKV1-39/1D-39 gene.

First, we used X-ray crystallography to determine the high-resolution crystal structures of the BCR Fab fragments from three subset #1 cases utilizing the IGHV1-2, IGHV1-3, and IGHV5-10 genes partnered with IGKV1-39/1D-39 to obtain information on the combining sites' structures and possible modes of intermolecular interactions that may mediate cell-autonomous signalling. Analysis of the experimental structures revealed that, despite the high sequence homology of the three proteins, the heavy and light chain CDR loops in the three BCR Fabs are arranged differently to shape binding cavities with distinct dimensions and polarity, thus suggesting that distinct antigens may be the physiological target of each subgroup. Unlike BCRs from other subsets previously characterised, the intermolecular contacts between the subset #1 Fab molecules in the three crystals involve diverse residues that are not indicative of a shared homotypic interaction. In addition, the surfaces involved in the contacts are small and the computed energies for the protein-protein contacts observed are suggestive at best of very weak interactions. In accordance to this, when we used analytical ultracentrifugation along with chromatographic and calorimetric methods to detect BCR Fab oligomerization in solution, no evidence of self-association was obtained, thus indicating that the receptors in solution either do not interact, or are characterized by self-dissociation constants that exceed

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the sensitivity limit of the techniques employed. To assess whether the cellular environment could promote self-association and signalling activity of the full-length BCR, we expressed subset #1 BCRs in the model triple knockout (TKO) pre-B-cell line, designed to measure Ca^{2+} ion influx using cytofluorimetry. All subset #1 BCRs tested were unable to mobilize autonomously the Ca^{2+} ions, despite being fully expressed on the cell surface and capable of signalling upon stimulation with anti-BCR antibody as a proxy for exogenous ligand. This result is in sharp contrast with the behaviour of all other CLL-derived BCRs so far characterised in this system that could activate a cell-autonomous signal as evidenced from a persistent Ca^{2+} ion influx in the transduced cells without requiring external stimuli. Hence, subset #1 BCRs differ from the so far characterized BCRs from CLL cases belonging to other stereotyped subsets, including clinically aggressive subset #2 and its satellite subset #169 as well as the clinically indolent stereotyped subset #4.
In conclusion, our combined biochemical and cellular analysis demonstrates that a constitutively active cell-autonomous signalling may not be a unifying feature of all CLL clones as previously proposed, and that other mechanisms may be at the roots of the BCR-mediated signal required for leukemic cell proliferation and development of CLL.

Disclosures Scarfo: *AbbVie:* Consultancy; *AstraZeneca:* Consultancy; *BeiGene:* Consultancy; *Janssen:* Consultancy; *Lilly:* Consultancy; *Octapharma:* Speakers Bureau. **Buske:** *Sobi:* Consultancy, Honoraria, Speakers Bureau; *Lilly:* Consultancy, Honoraria, Speakers Bureau; *MSD:* Research Funding; *Amgen:* Research Funding; *Bayer:* Research Funding; *BeiGene:* Consultancy, Honoraria, Speakers Bureau; *Novartis:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Incyte:* Consultancy, Honoraria; *Abbvie:* Consultancy, Honoraria, Speakers Bureau; *Gilead Sciences:* Consultancy, Honoraria, Speakers Bureau; *Celltrion:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *MorphoSys:* Consultancy, Honoraria, Speakers Bureau; *Regeneron:* Consultancy, Honoraria; *Janssen:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Roche/Genentech:* Consultancy, Honoraria, Research Funding, Speakers Bureau. **Ghia:** *Lilly/Loxo Oncology:* Consultancy, Honoraria, Research Funding; *MSD:* Consultancy, Honoraria, Research Funding; *Janssen:* Consultancy, Honoraria, Research Funding; *Roche:* Consultancy, Honoraria, Research Funding; *BeiGene:* Consultancy, Honoraria, Research Funding; *AstraZeneca:* Consultancy, Honoraria, Research Funding; *AbbVie:* Consultancy, Honoraria, Research Funding; *BMS:* Consultancy, Honoraria, Research Funding.

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