P2RX5 is a human B-cell marker and a multi-lineage immunotherapy target for lymphoid and plasma cell malignancies

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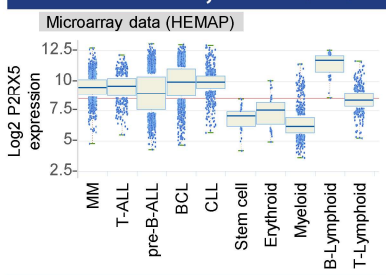
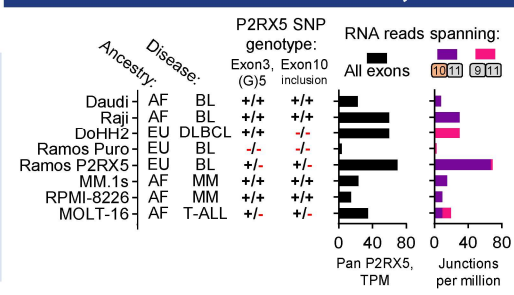
Many successful therapies for the treatment of B-cell neoplasms target B-cell lineage-restricted markers such as CD19 and BCMA. Nevertheless, relapse due to antigen escape and on-target-off-tumor toxicities still occur, highlighting the need for novel targets. Here, we identify purinergic receptor 5 (P2RX5), an ATP-gated ion channel, as a B-cell marker and a immunotherapy target for lymphoid and plasma cell malignancies

1. P2RX5 is upregulated in ALL, CLL, NHL and myelomas.

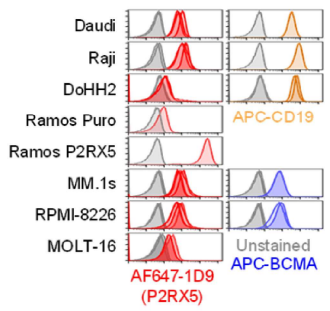


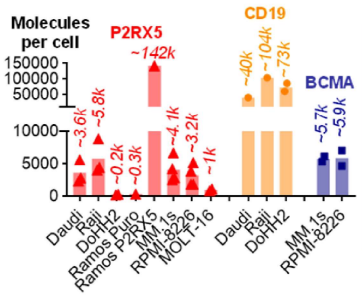
← **Fig. 1:** P2RX5 expression in curated microarray data across 36 hematological malignancies (HEMAP).

5. The custom mAbs, 1D9 and 2F4, detect P2RX5-FL in B-cells, ALL, NHL and myelomas.



← **Fig. 5A:** RNA sequencing data of P2RX5 in cell lines.





← **Fig. 5B:** Live cell flow cytometry of cell lines using a P2RX5-directed mAb, clone 1D9.

2. In normal human tissues, P2RX5 is only expressed in B-cells.

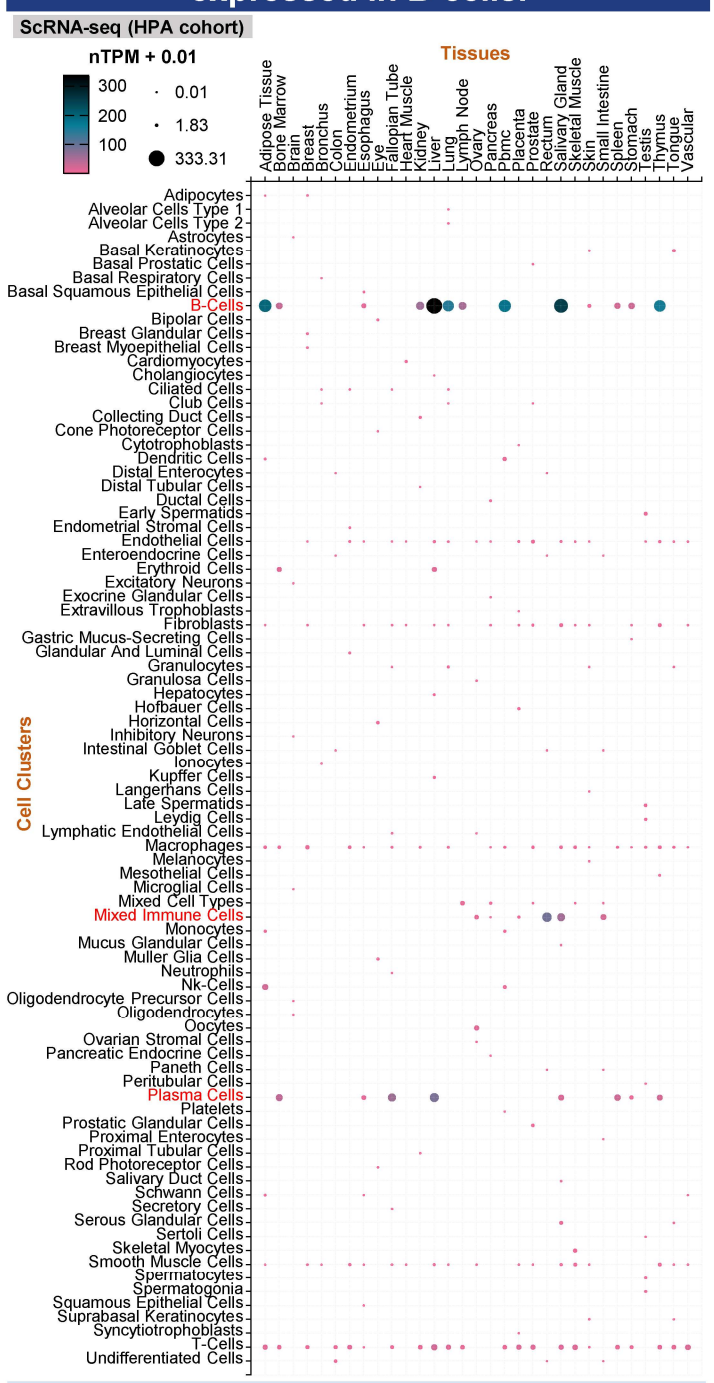


Fig. 2: Single cell sequencing data of P2RX5 in 31 human tissues from The Human Protein Atlas.

4. P2RX5-FL genotyped MM pts have worse outcomes

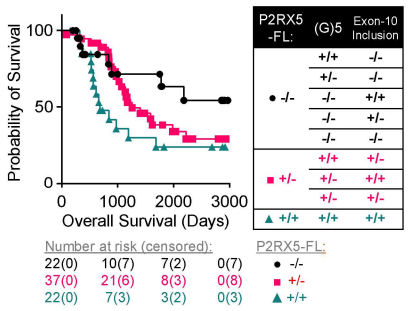
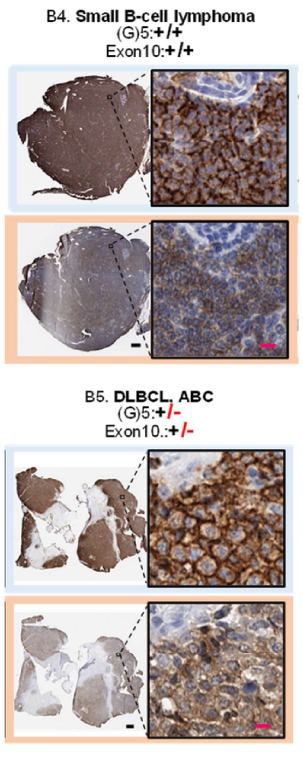


Fig. 4: Treatment responses of patients with multiple myeloma (MM), specifically those of African ancestry (COMMPASS dataset), as stratified base on their P2RX5-FL status.



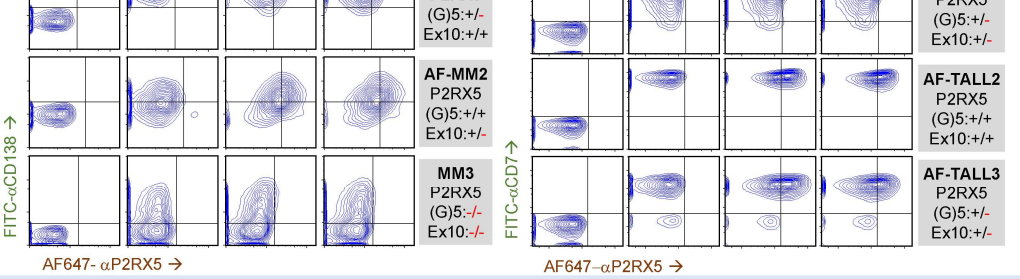


Fig. 5C: Live cell flow cytometry of MM and T-ALL samples using P2RX5-directed mAbs, clones 1D9 and 2F4.

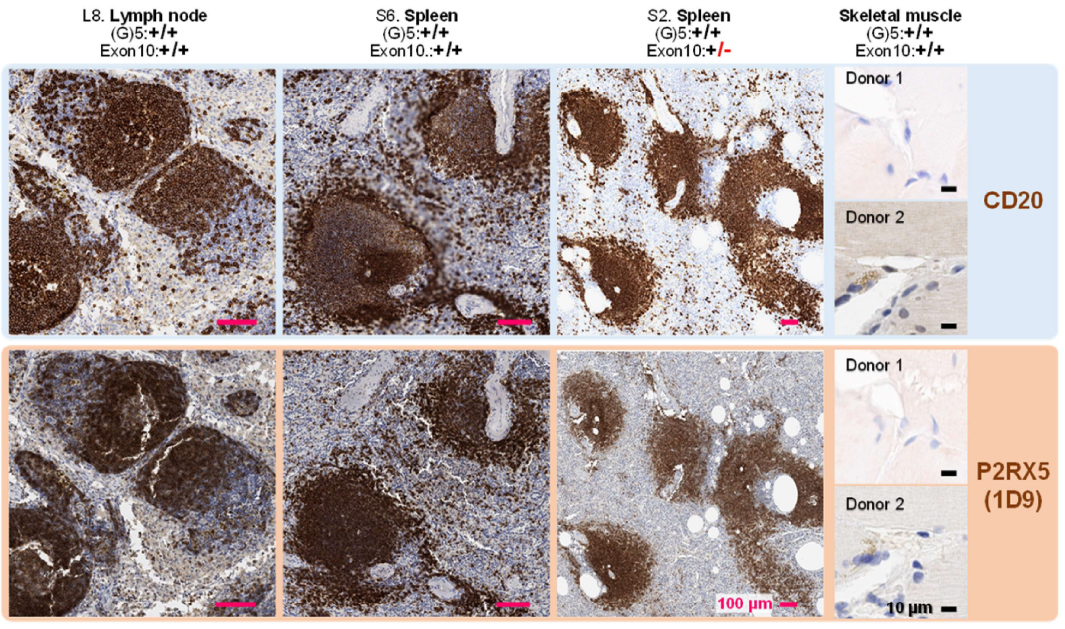


Fig. 5D: FFPE IHC. P2RX5-directed mAb (clone 1D9) and CD20 staining in DAB (brown). Blue nuclear counterstain. P2RX5 genotype was determined from RNA and DNA analysis.

6. P2RX5-directed immunotherapies are specific and potent.

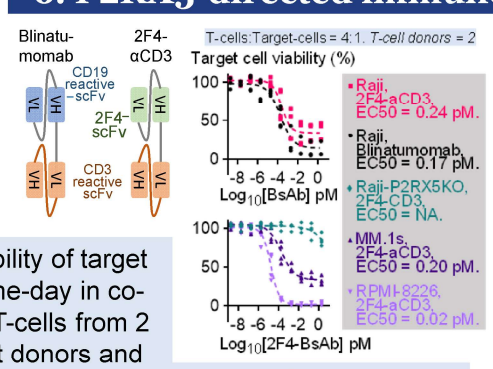


Fig. 6a: Viability of target cells after one-day in co-culture with T-cells from 2 independent donors and the indicated concentration of BsAb. Cell viability is shown relative to that of BsAb-untreated controls.

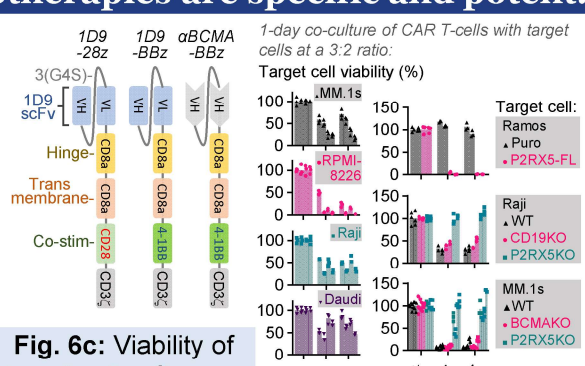


Fig. 6c: Viability of target cells after one day in coculture with various 2F4-equipped CAR T-cells. Cell viability is shown relative to that of cells co-cultured with mock-transduced T-cells. Each dot represents a technical replicate while each bar is data from a unique donor.

3. Due to deleterious alleles, only donors of African ancestry express full-length P2RX5 (P2RX5-FL).

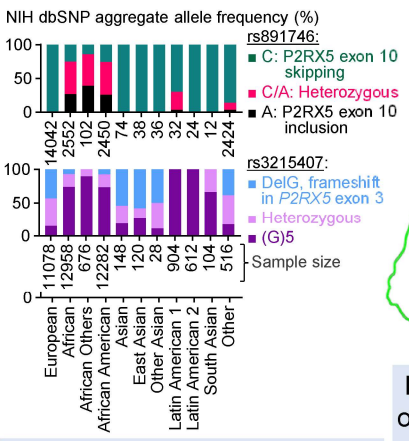


Fig. 3A: Frequencies of deleterious P2RX5 alleles from the NCBI dbSNP.

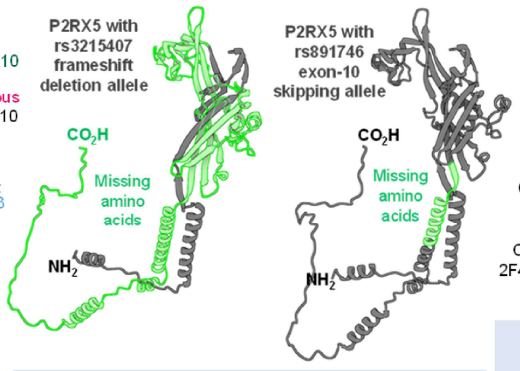


Fig. 3B: AlphaFold predicted structure of P2RX5 with missing amino acids due to deleterious alleles highlighted in green. Image generated by molstar.

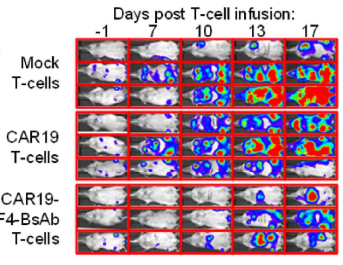


Fig. 6b: Bioluminescent imaging of NSG mice with established luc+ Raji CD19 KO cell xenografts and treated with $3x10^5$ 2F4-αCD3-secreting CAR19 T-cells.

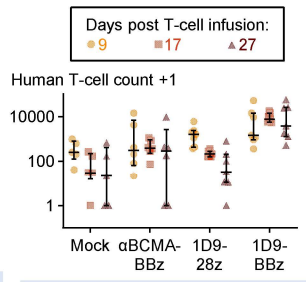


Fig. 6d: Expansion of various CAR T-cells in mice with established RPMI-2886 xenografts post-injection.

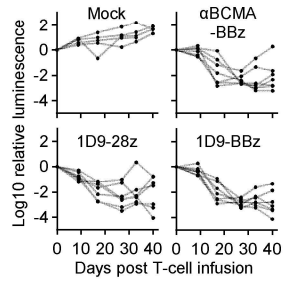


Fig. 6e: Bioluminescent imaging orthotopic MM xenografts (established via intratibial injections of RPMI-8226) during treatment with P2RX5-directed CARs.