

Integrated single-cell transcriptomic and flow cytometric profiling identifies candidate immunotherapeutic targets and their modulation by ibrutinib in Waldenström macroglobulinemia

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Background

Waldenström macroglobulinemia (WM) comprises clonally related malignant B cells and plasmacytic tumor cells with distinct differentiation states. These compartments may display different and partially overlapping surface-antigen profiles, potentially limiting the efficacy of single-target cellular or immune therapies. Although ibrutinib provides durable clinical benefit, residual disease and treatment resistance remain common, and its effects on candidate immunotherapeutic targets are incompletely understood. We characterized target expression across malignant WM compartments, evaluated its modulation during ibrutinib therapy, and validated selected targets at the protein level.

Methods

Single-cell transcriptomic profiles of bone marrow B-lineage cells from patients with WM were analyzed to identify malignant B-cell and malignant plasma-cell populations. Expression of candidate targets, including CD19, CD20, FCRL5, BCMA, CD138, CD70, and ROR1, was compared between compartments. Longitudinal samples collected at baseline and during ibrutinib treatment were used to assess treatment-associated changes and differences between clinical responders and non-responders. Multiparameter flow cytometry was subsequently performed on bone marrow samples from six WM patients to compare surface-protein expression between CD19⁺CD138⁻ B-cell-like and CD19⁺CD138⁺ plasma-cell-like populations.

Results

Single-cell analysis demonstrated distinct but partially overlapping target profiles. CD19 RNA was expressed in both malignant B cells and malignant plasma cells, with higher expression in malignant B cells. CD20 was predominantly expressed in malignant B cells, whereas BCMA and CD138 were enriched in malignant plasma cells. FCRL5 RNA was highly expressed in both compartments. Following ibrutinib treatment, CD19 RNA decreased significantly in malignant B cells and malignant plasma cells ($P=0.0018$ and $P=0.0061$), as did FCRL5 RNA ($P=4.58 \times 10^{-5}$ and $P=0.0010$). BCMA RNA remained relatively stable, whereas CD138 increased in malignant plasma cells ($P=0.030$). CD20 expression in malignant B cells was unchanged overall but was higher in non-responders than responders ($P=4.40 \times 10^{-4}$); BCMA expression in malignant B cells was also higher in non-responders ($P=0.035$). CD70 RNA was primarily expressed in malignant B cells and remained stable during treatment.

Flow cytometry confirmed CD19 protein expression in both B-cell-like and plasma-cell-like

populations, although expression was lower in the latter. CD20 protein was expressed on B cells but was nearly absent from plasma cells. BCMA protein was primarily expressed on plasma cells, while B-cell expression was heterogeneous. Despite high FCRL5 RNA abundance, FCRL5 protein levels were low in both populations. ROR1 showed low RNA and protein expression. CD70 demonstrated discordant RNA and protein distributions: RNA was mainly detected in malignant B cells, whereas protein was mainly detected in plasma cells.

Conclusions

WM malignant B-cell and plasma-cell compartments exhibit complementary but incompletely overlapping antigen profiles, making complete eradication with a single target unlikely. CD19 may provide partial cross-compartment coverage but decreases following ibrutinib. CD20 primarily targets B cells, whereas BCMA primarily targets plasma cells, supporting CD20 plus BCMA as a rational dual-target strategy. The discordance between RNA and surface-protein expression for FCRL5 and CD70 highlights the need for patient-specific protein validation before target selection. Therapeutic strategies should therefore consider antigen density, cellular heterogeneity, and prior ibrutinib exposure.

482/500 words

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