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HiFiBiO THERAPEUTICS

A Novel Biparatopic KLRG1 Antibody Selectively Depletes Effector Memory CD8+ T Cells for Autoimmune Disease Treatment

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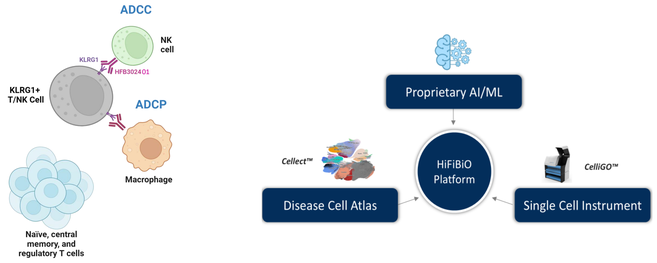
BACKGROUND

Cytotoxic CD8+ T cells contribute to multiple inflammatory and autoimmune diseases through tissue damage and destruction. KLRG1 (Killer cell Lectin-like Receptor G1) marks highly cytotoxic effector memory (TEM) and effector memory CD45RA+ (TEMRA) CD8+ T cell subsets that are expanded in the inflamed tissues or peripheral blood of autoimmune disease patients. Selective depletion of KLRG1+ CD8+ T cells is an attractive therapeutic approach that could offer lasting clinical benefits in selected autoimmune disease patients without global immune suppression.

Using our proprietary single cell microfluidic platform, we identified a series of anti-human KLRG1 antibodies with high binding affinity and functional activity. Selected binding domains were used to design a biparatopic antibody, HFB302401, with single-digit nanomolar affinity to human and cynomolgus KLRG1 and capable of inducing more potent ADCC and ADCP of KLRG1+ cells than a mono-specific benchmark in vitro. HFB302401 selectively depleted CD8+ TEM and TEMRA subsets from PBMCs collected from healthy donors and autoimmune patients, sparing other T cells. HFB302401 also demonstrated potent and sustained depletion of KLRG1+ KG1 cells in vivo.

HiFiBiO's Drug Intelligent Science (DIS®) single-cell immune profiling platform provides insights into the abundance and activity of immune cell subsets in particular disease contexts, allowing precise targeting of pathogenic cell populations that drive autoimmunity in individual patients. Based on its potent pharmacological activity and favorable developability profile, HFB302401 is currently being developed as a novel immunotherapy for cytotoxic T cell-mediated autoimmune diseases coupled with a DIS®-enabled patient biomarker strategy.



RESULTS: Target analysis

Single cell analysis of KLRG1 in HiFiBiO Disease Cell Atlas (Cellect™)

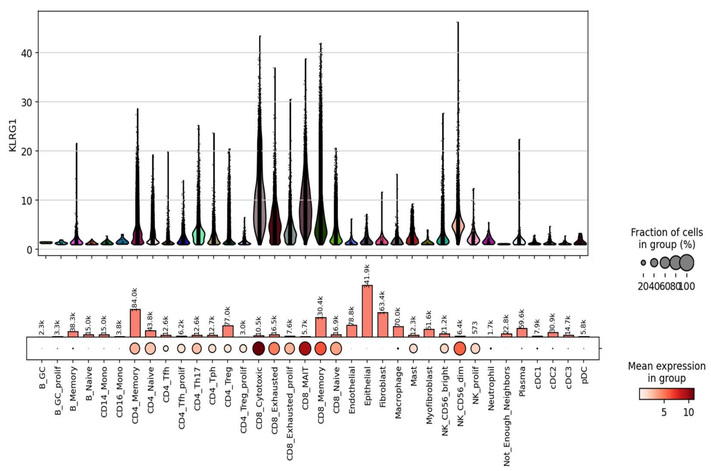


Figure 1. ScRNA-seq analysis of 2.4M cells from tissues collected from I&I patients reveals KLRG1 main expression in CD8+ T and CD56 dim NK cells.

RESULTS: Antibody characterization

Discovery of anti-KLRG1 antibodies with different epitopes

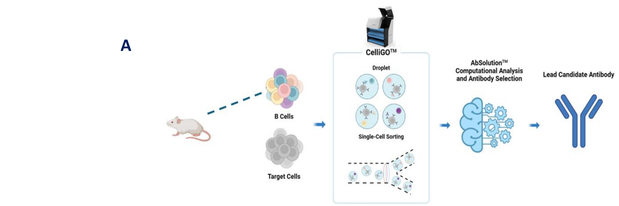


Figure 2 (A) Discovery of anti-KLRG1 antibodies using HiFiBiO single cell microfluidic platform CelliGO™ Mice were immunized with recombinant human KLRG1 ECD. Spleens were harvested and processed into single-cell suspensions. Using the CelliGO microfluidic platform, individual cells were screened for IgG affinity to hKLRG1. IgG variable regions from selected cells were sequenced, cloned, and expressed as recombinant antibodies. **(B) Epitope binning of anti-KLRG1 antibodies by competitive binding.** HFB02050 and HFB02044 were selected as representative of two groups of antibodies binding to non-overlapping epitope bins and engineered as part of a bi-paratopic antibody for optimal binding and ADCC profiles

In vitro characterization of biparatopic Ab HFB302401

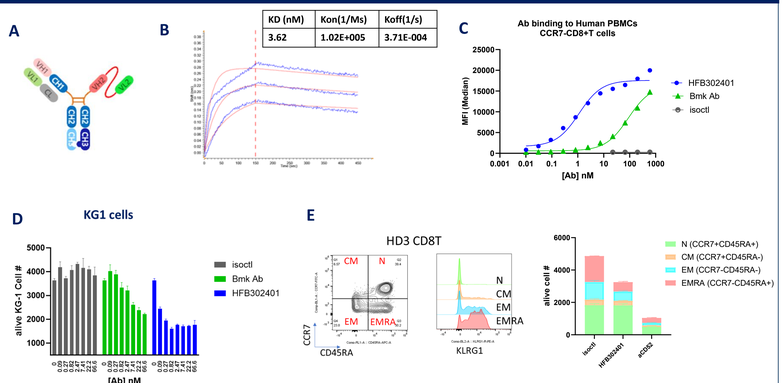


Figure 3 (A) Biparatopic Ab configuration. **(B)** KD values of HFB302401 antibody binding to hKLRG1-ECD by Octet. **(C)** Cellular binding of HFB302401 to CCR7-CD8+ T cells in human PBMCs compared to benchmark (Bmk) and isotype control (isoctl) antibodies. **(D)** ADCC killing activity of HFB302401 using KLRG1+ KG1 cells as target cells and allogenic NK cells as effector cells. E:T=5:1. **(E)** Selective depletion of KLRG1+ CD8+TEM and TEMRA cells in healthy donor (HD) PBMCs. PBMCs were incubated with 100nM Ab for 72h. aCD52: alemtuzumab biosimilar; N:naïve; CM: central memory; EM: effector memory; EMRA: effector memory CD45RA+

PD depletion of KLRG1 expressing human cells in mice

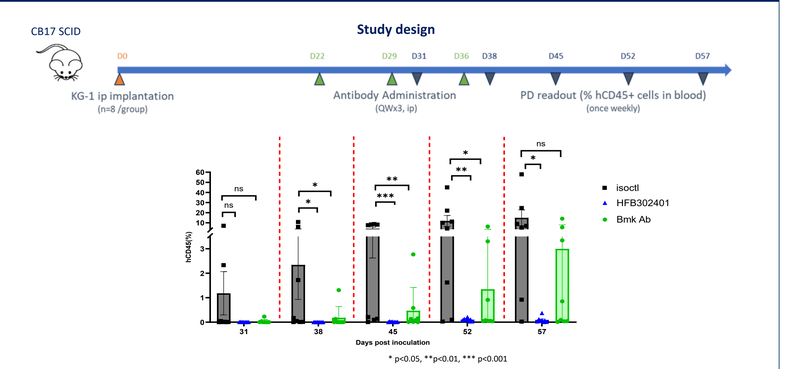


Figure 4 In vivo Depletion of KG-1 Cells Implanted in CB17 SCID Mice Administration of HFB302401 (QWx3) results in profound and sustained depletion of KG1 cells in the blood of CB17 SCID mice

RESULTS: Translational analysis

Ex vivo study of HFB302401 with I&I patient PBMCs

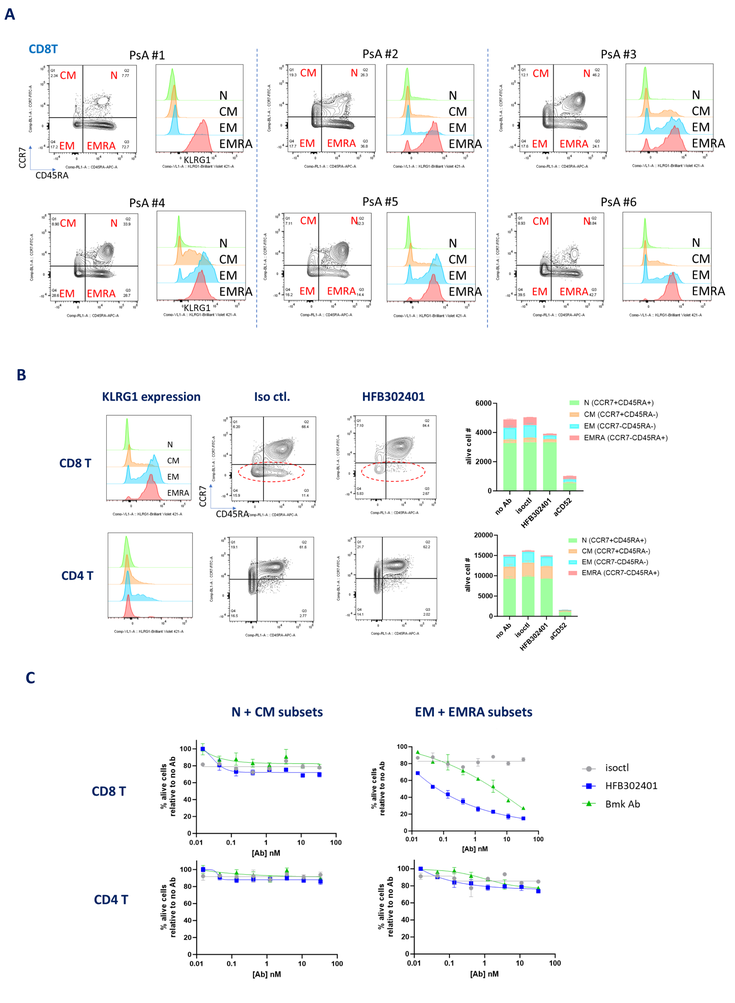


Figure 5 (A) Flow cytometry analysis of KLRG1 in I&I patient PBMCs (n=6). KLRG1 is predominantly expressed on CD8+TEM & TEMRA population in psoriatic arthritis (PsA) patient PBMCs. **(B)** HFB302401 selectively depletes KLRG1+ CD8+ TEM and TEMRA cells, sparing naïve and central memory CD8T and CD4 T cells. PsA patient PBMCs were incubated with 33.3nM Ab for 72h. **(C)** HFB302401 depletes KLRG1+ CD8+ TEM and TEMRA cells more potently than benchmark antibody. N:naïve; CM: central memory; EM: effector memory; EMRA: effector memory CD45RA+

Conclusion

We have discovered anti-KLRG1 antibodies capable of depleting KLRG1+ cells through ADCC/ADCP and selected a clinical candidate with biparatopic design, HFB302401, for further development. Highlight of HFB302401:

- *A humanized anti-KLRG1 biparatopic mAb binding specifically to human/cyno BTLA with single-digit nanomolar affinity and potent ADCC/ADCP depletion activity*
- *Exhibit potent and selective depletion of KLRG1+ CD8+ TEM and TEMRA, while sparing other T cell populations.*
- *Superior binding and depletion activity in vitro and in vivo than a clinical-stage benchmark Ab*
- *Favorable developability and pharmacokinetic profiles*
- *Innovative translational strategy applying HiFiBiO's Drug Intelligent Science (DIS®) single-cell immune profiling platform for indication selection*
- *HFB302401 IND filing is anticipated in the second half of 2026*

HiFiBiO THERAPEUTICS

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