Poster 31
CIS 2025 Annual Meeting
May 2nd, 2025

CXCR5 as a Therapeutic Target in Autoimmune Diseases: Insights from Sjögren's Disease

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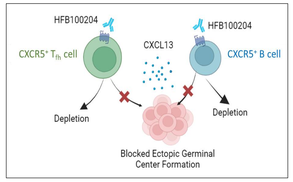
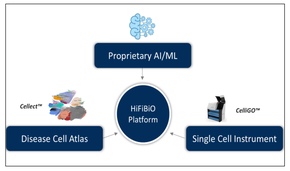
BACKGROUND

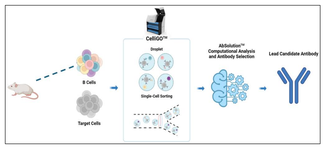
- CXCR5 is a seven-transmembrane G(i)-protein-coupled receptor predominantly expressed on mature resting B cells, tonsillar B cells, and follicular helper T cells (T_{fh}). Its interaction with the ligand CXCL13 promotes cell migration and contributes to the formation of ectopic germinal centers (eGCs).
- Aberrant expression and signaling of CXCL13 and/or CXCR5 have been linked to autoimmune and chronic inflammatory diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren's disease (SD). Given the critical role of B cells and T_{fh} cells in these immune-mediated conditions, we propose that selective depletion of pathogenic CXCR5$^+$ cells via ADCC could offer a promising therapeutic strategy for autoantibody-driven I&I disorders.
- SD, a common systemic autoimmune disease, is characterized by the infiltration of lymphocytes into the salivary and lacrimal glands and the production of autoantibodies against soluble nuclear antigens. SD progress through different stages, the most advanced stage being characterized by malignant transformation into lymphoma.
- We developed HFB100204, a Fc-enhanced monoclonal antibody that depletes pathogenic CXCR5$^+$ cells via ADCC and blocks CXCL13-dependent signaling. Using HiFiBiO Therapeutics' translational single-cell platform, we profiled CXCR5$^+$ immune cells in SD patients across disease stages and assessed HFB100204-mediated CXCR5$^+$ cell depletion both *in vitro* and *in vivo*.





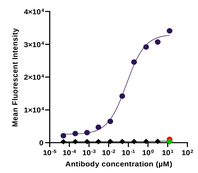
RESULTS: Antibody characterization

HFB100204 discovery and characterization



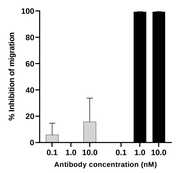
HFB100204 discovery workflow using HiFiBiO single cell microfluidic platform

Mice were repeatedly immunized with human CXCR5 cDNA. Spleens and lymph nodes were harvested and processed into single-cell suspensions. Using the CelliGO microfluidic platform, individual cells were screened for IgG affinity to hCXCR5 using engineered cells. IgG variable regions from selected cells were sequenced, cloned, and expressed as recombinant antibodies.



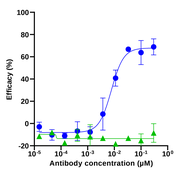
HFB100204 binds hCXCR5

Flow cytometry binding assay of HFB100204 on wild type (●), and hCXCR5-KO Raji cells (◆). Isotype controls at highest concentrations shown: WT (●) and KO (◆)



HFB100204 inhibits CXCL13-driven CXCR5$^+$ cell migration

In a transwell migration assay, HFB100204 (black) completely inhibited M300 hCXCR5 cell migration at concentrations above 1 nM, compared to the isotype control (grey)

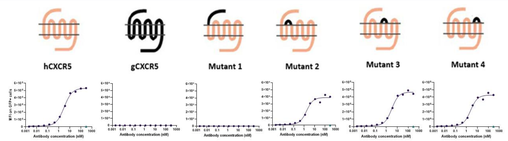


HFB100204 inhibits CXCL13-induced receptor activation

HFB100204 (●) efficiently reverses hCXCL13-induced suppression of intracellular cAMP production, compared to an isotype control (▲)

RESULTS: HFB100204 characterization (continued)

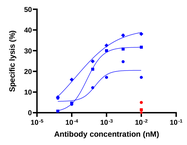
Epitope mapping



HFB100204 binds to the N-Term extracellular tail of hCXCR5

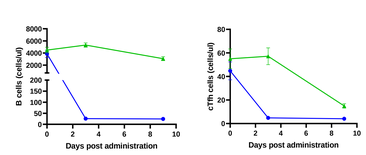
Four chimeric mutants were made by swapping hCXCR5's external domains with goat CXCR5 (gCXCR5) sequences. Isotype control at highest concentration is shown (▲).

Depletion of CXCR5$^+$ cells in vitro and in vivo



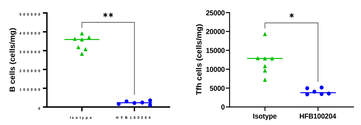
Depletion of hCXCR5$^+$ primary B cells *in vitro*

ADCC potency of HFB100204 (blue) vs. isotype control (red) on primary B cells from 3 donors.



Depletion of hCXCR5$^+$ blood cells *in vivo*

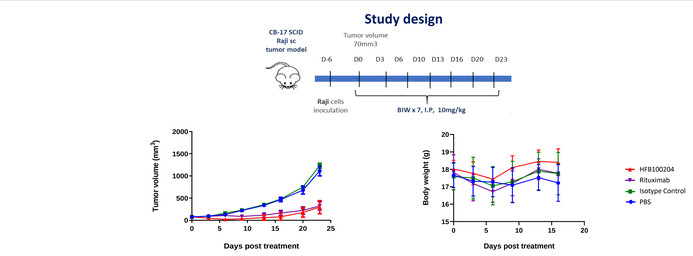
PD evaluation of HFB100204 (mIgG2a) in hCXCR5-KI mice. 8 mice (4 male and 4 female) received one injection of 10 mg/kg of HFB100204 (●) or 10 mg/kg of an isotype control (▲). Blood samples were collected 1-, 3- and 9-days post-injection. Number of CXCR5$^+$ B cells was evaluated by flow cytometry.



Depletion of hCXCR5$^+$ spleen cells *in vivo*

At sacrifice (9 days), spleen from the 8 hCXCR5-KI mice who received HFB100204 (●) or an isotype control (▲) were recovered and dissociated. Number of CXCR5$^+$ B and T_{fh} (CD3$^+$ CD4$^+$ CD95$^+$ CXCR5$^+$) cells was evaluated by flow cytometry.

PD depletion of CXCR5 expressing human cells demonstrated in mice



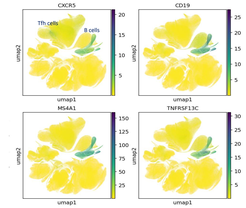
Anti-xenograft efficacy in subcutaneous Raji xenograft model

Administration of HFB100204 results in potent anti-tumor activity (graph on the left), with tumor growth inhibition (TGI) of 76% at the end point and significantly higher TGI than Rituximab until day 23 . It is well tolerated and doesn't result in any significant weight loss (graph on the right). Xenograft volumes and body weight were monitored twice per week after randomization.

RESULTS: Translational analysis

Rationale for targeting CXCR5 over other B cell depletion targets

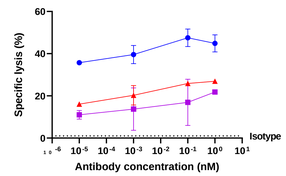
Analysis of our proprietary disease cell atlas, a single cell transcriptomic database including 15M+ annotated cells across 100+ indications, from tissues collected from I&I patients and healthy individuals:



Targeting CXCR5$^+$ cells has the potential to eliminate circulating B cells and disrupt eGCs

In contrast to B cell-depleting strategies that target CD20 or BAFFR, a depleting antibody targeting CXCR5 holds the potential to eliminate T follicular helper (T_{fh}) cells, which are critical for the initiation and maintenance of eGCs in autoimmune diseases.

Comparison with SOC:



HFB100204 has superior depleting activity over anti-CD20 and anti-BAFFR based therapies

B (Target) cells from 2 donors were cultured with activated NK (Effector) cells from 1 donor in the presence of either HFB100204 (●), Ianalumab biosimilar (▲), or Rituximab biosimilar (■) at different Ab concentrations.

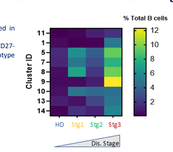
Single cell analysis of PBMC from SD patients at different disease stages

Insights from single B cell profiling on samples from SD patients at different stages of the disease

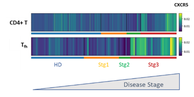
B cell clusters (16 integrated samples, 29 clusters)

Selection of B cells clusters enriched in patients compared to HD.
Selected B cells are CD21-/low, CD27-/low, a pathogenic B cell phenotype associated with disease activity.

Relative abundance of expanding B cell clusters with disease stage



CXCR5 expression in T_{fh} cells



Analysis performed on isolated B cells from PBMC from 4 healthy donors (HD) and 4 patients for each stage of the disease (Stg1, 2 and 3) reveal that:

- Certain pathogenic CXCR5$^+$ B cell clusters (CD21-, CD27-) expand as the disease progresses through its stages
- CXCR5 expression levels in T_{fh} cells increase as the disease progresses through these stages

SUMMARY

- We identified HFB100204, a high-affinity CXCR5 inhibitor that exhibits sub-nanomolar ADCC activity against both B and T_{fh} cells and achieves superior B cell depletion in vitro compared to anti-CD20 and anti-BAFFR antibodies, which are under clinical investigation for SD. It also blocks CXCL13-induced signaling and B cell migration.
- By simultaneously targeting migration and survival of CXCR5$^+$ B and T_{fh} cells, both central players in eGC formation and disease persistence, HFB100204 offers a distinct advantage over existing therapies. This dual mechanism may reduce pathogenic autoantibody production, tissue damage, and the likelihood of relapse, positioning it as a promising next-generation treatment for SD.
- Single cell insights of SD patient samples provided strong rationale targeting CXCR5 expressing cells in the treatment of SD, especially moderate to severe stage SD, where there is a clear unmet medical need.

Acknowledgement

We would like to thank Prof. David Saadoun, Paul Regnier, and Cindy Marques (Département de médecine interne et immunologie clinique, hôpital Pitié-Salpêtrière) for collaboration and for providing the SD patient samples.

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