Characterization of anti-C5aR antibodies for specific targeting of myeloid cells and neutrophils in the TME

Olivier Demaria, Lucie Rubio, Nourhene Belaid, Guillaume Habib, Cécile Bonnafous, Robert Zerbib, Mathieu Blery, Yannis Morel, Joanna Fares - Innate Pharma, 117 Avenue de Luminy, 13009 Marseille, France

Background

Accumulation within tumors of immunosuppressive myeloid cells and neutrophils is associated with poor prognosis in many cancers, as well as resistance to checkpoint blockade. A hallmark mechanism of synergy in immunotherapy is the elimination of these immunosuppressive cells to allow for the reactivation of effector cells. From a therapeutic perspective, we aimed to specifically target these suppressive mediators to impede their recruitment into the tumor microenvironment (TME) and promote a more potent antitumor response.

We confirm here the distinctive restricted expression profile of human C5aR1 on circulating myeloid-derived suppressor cells (MDSCs), which produce less immunosuppressive cytokines, and are also functionally unable to suppress T and NK cells.

We present in mice the anti-tumor efficacy of targeting the C5a/C5aR1 pathway in combination with PD-1 iabnodies.

Selective expression of C5aR1 on cells of the myeloid lineage

C5aR1 blockade synergizes with anti-PD-1 blockade to delay tumor progression

Mechanism of action

Combination therapy with anti-C5aR1 and anti-PD-1

Conclusions

- C5aR1 is selectively expressed on circulating human neutrophils and myeloid cell subsets.
  - In tumor-bearing mice, C5aR1 is strongly expressed in both tumor infiltrating and circulating suppressive myeloid populations. C5aR1 expression is further upregulated compared to tumor-free animals.
  - C5aR1 expression is higher in human M2-derived immunosuppressive cells compared to the non suppressive M1 subset.
  - Accordingly to their high expression level of C5aR1, M2-derived macrophages present a stronger ability to migrate to C5a, suggesting a role for C5aR1 in the chemotaxis of myeloid cells to the tumor microenvironment.
  - IPH5401, a fully humanized anti-C5aR1 antibody, effectively inhibits the C5a-mediated effects on neutrophil activation and migration.
  - The combined administration of a surrogate anti-C5aR1 blocking antibody with anti-PD-1 synergistically reduced tumor growth in a poorly infiltrated tumor model in vivo.

Overall, these data provide a strong incentive to clinically explore combination therapies with anti-PD-1 using a C5aR1 antibody. IPH5401 represents a unique opportunity to successfully reverse the tumor immunosuppressive microenvironment and overcome tumor resistance in cancer immunotherapy.

Immunosuppressive human M2 macrophages express high levels of C5aR1 and respond strongly to C5a-induced chemotaxis

A) Nonspecific C5a activates human peripheral blood monocytes and neutrophils. This activation is strongly mediated by C5aR1.

B) C5aR1 blockade decreases MDSC infiltration and their immunosuppression of effector cells.

C) C5aR expression in the myeloid and the lymphoid populations of peripheral blood of healthy donors analyzed by FACS. Representative histograms from Donor DS271 are shown here. Black lines represent FMO staining.

D) Combination therapy with anti-C5aR1 and anti-PD-1 significantly impaired tumor growth. A) C5aR expression in the myeloid and the lymphoid populations of peripheral blood of healthy donors analyzed by FACS. Representative histograms from Donor DS271 are shown here. Black lines represent FMO staining.

E) Anti-PD-1 blocking mice show increased migration of M2-derived macrophages compared to M1-derived macrophages in a transwell assay. M2 cells were analysed with Cell Titer Glo in the lower chamber.

F) Anti-C5aR1 treatment also decreases M2 migration to C5a compared to M1-derived macrophages.

G) Anti-PD-1-treated mice show a stronger ability to migrate to C5a, suggesting a role for C5aR1 in the chemotaxis of myeloid cells to the tumor microenvironment.

H) IPH5401 selectively inhibits C5a-induced activation and migration of human neutrophils.

A) Neutrophil activation by C5a evaluated by expression levels of CD11b and CD62L markers.

B) Neutrophil migration to C5a in a transwell assay. Controls include other chemotactic agents such as IL8 and CCL2.

C) Migrating cells were analyzed with Cell Titer Glo in the lower chamber.

D) FACS analysis of C5aR1 expression in subcutaneously engrafted A20 tumors.