IPH5401 anti-human C5aR1 antibody targets suppressive myeloid cells in the TME

Poster #P268

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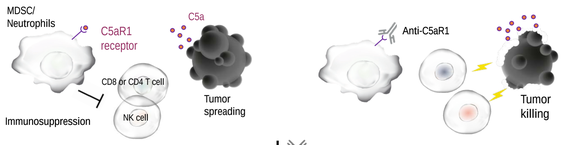
Background

The elimination of immunosuppressive cells, such as myeloid cells and neutrophils, to allow the reactivation of effector cells, is a hallmark of synergy in immunotherapy. Indeed, these immunosuppressive cells are associated with a poor prognosis in many cancer types, and resistance to checkpoint blockade. For therapeutic purposes, we aimed to target the recruitment of these major mediators of protumoral inflammation in the tumor microenvironment (TME) specifically. The complement system is a network of more than 50 different plasma and membrane-associated proteins. This component of the innate immune system plays a key role in host defense against pathogens and in tissue homeostasis. C5 cleavage during complement activation generates the anaphylatoxin C5a. C5a is a highly potent chemoattractant that induces immune cell activation and recruitment to inflamed tissues. The immune cells recruited include neutrophils, eosinophils, monocytes, basophils, and mast cells. C5a binds the seven-transmembrane span receptors C5aR1 (CD88) and C5aR2 (C5L2). C5aR1 blockade is thus a potentially powerful means for controlling myeloid suppressive cells in the TME. We developed IPH5401, a fully human monoclonal anti-C5aR1 blocking antibody that prevents binding to C5a. C5aR1 is upregulated in patients with NSCLC displaying progression after an initial response to anti-PD-(L)1 therapy (IO) [1].
We first explored the expression profile of C5aR1 in the TME further, focusing on both mRNA and protein levels, in several solid cancers displaying various levels of infiltration with C5aR1-positive immune cells. We then demonstrated that IPH5401 blocked the activation and migration of human neutrophils *in vitro*.
These results support our ongoing multicenter, open-label, dose-escalation and dose-expansion Phase I clinical trial (STELLAR-001) evaluating the safety and efficacy of IPH5401 in combination with durvalumab, an anti-PD-L1 immune checkpoint inhibitor, as a treatment for patients with advanced solid tumors [1].

Mechanism of action

FIGURE 1: IPH5401 – First-In-Class Anti-C5aR1 mAb



C5a stimulates suppressor cell recruitment and activation, thereby inhibiting immune effector cells

Inhibition of C5aR1 signaling increases CD8 T-cell infiltration and function

IPH5401 anti-C5aR1efficacy *in vitro*

FIGURE 4A: IPH5401 inhibits C5a-induced CD11b expression on neutrophils *in vitro*

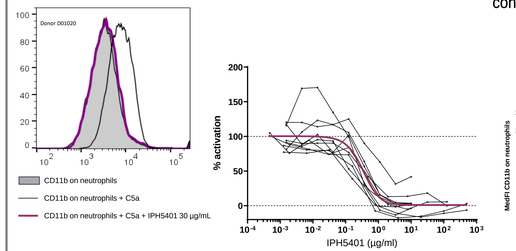
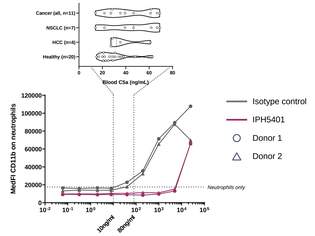
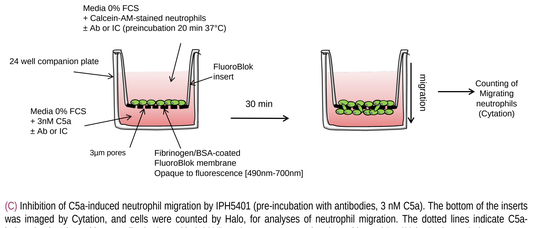


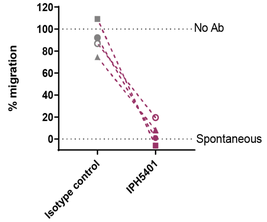
FIGURE 4B: IPH5401 efficiently blocks the CD11b expression induced by supra-physiological concentration of C5a *in vitro*



(A, B) Whole blood from healthy volunteers (BIO-002-IPH study. A: n=10, B: n=2) was incubated with IPH5401 or isotype control (A: at indicated concentrations, B: 10 µg/mL), before stimulation with C5a (A: 3 nM, B: at indicated concentrations) for 20 min at 37°C. CD11b surface expression on neutrophils was then quantified by flow cytometry. (A) The purple line on the right panel is the fitted curve for the 10 samples analyzed, giving a mean EC50 of 0.33 µg/mL ± 0.15 (SD). (B) Upper panel, C5a concentrations in plasma from healthy donors (HD), hepatocellular carcinoma (HCC) patients and non-small cell lung carcinoma (NSCLC) patients, determined by ELISA.

FIGURE 4C: IPH5401 inhibits C5a-induced neutrophil migration *in vitro*

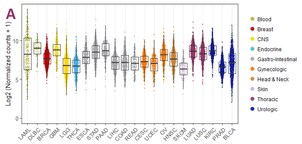




(C) Inhibition of C5a-induced neutrophil migration by IPH5401 (pre-incubation with antibodies, 3 nM C5a). The bottom of the inserts was imaged by Cytation, and cells were counted by Halo, for analyses of neutrophil migration. The dotted lines indicate C5a-induced migration without antibody (No Ab) (100%) and spontaneous migration without C5a (0%). Each symbol represents neutrophils isolated from an independent donor.

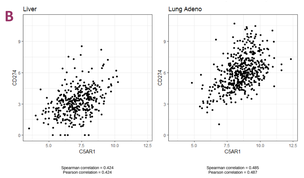
C5aR1 mRNA levels

FIGURE 2A: The C5aR1 transcript is found in all primary tumors (TCGA)



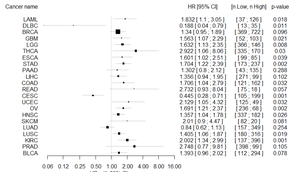
(A) Expression of C5AR1 in primary tumors according to The Cancer Genome Atlas (TCGA) RNA sequencing (RNAseq) data. Data were obtained with the tcgabiolinks package.
Cancer abbreviations: BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma.

FIGURE 2B: Levels of the PD-L1 and C5aR1 mRNAs are poorly correlated in primary lung adenocarcinoma and liver cancer (TCGA)



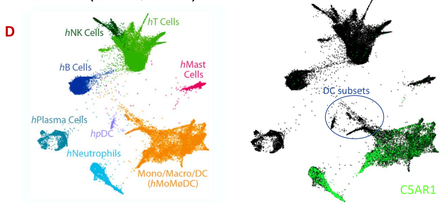
(B) Comparison of the levels of C5AR1 and CD274 (PD-L1) mRNA in primary lung adenocarcinoma and primary liver hepatocellular carcinoma, according to TCGA RNAseq data

FIGURE 2C: C5aR1 expression in primary tumors is associated with poor clinical outcome (TCGA)



(C) Forest plot showing the impact of C5AR1 levels on survival in the TCGA cohort for different types of cancer. Thresholds separating high- and low-expression groups for each cancer type were selected so as to minimize the p-value. This analysis required each group to contain at least 20% of the patients. The HR and its confidence interval; the number of patients in high-/low-expression groups and the p-values are indicated on the right.
HR: Hazard Ratio. CI: Confidence interval. Cancer abbreviations as in (A).

Figure 2D: scRNAseq showing that lung tumor-infiltrating myeloid cells, except DC, contain C5aR1 mRNA (Zilionis, 2019 [2])



(D) Two-dimensional visualization (SPRING plots) of single-immune cell transcriptomes (n = 34450) for patient lung tumor biopsies (n = 7). Data from Zilionis et al., 2019 [2]. Immune clusters are shown on the left and C5AR1 mRNA levels are shown in green on the right.
Figure from https://kleintools.hms.harvard.edu/tools/springViewer_1_6_dev.html?datasets/Zilionis2019/human/NSCLC_immune

C5aR1 protein levels

FIGURE 3A: C5aR1 protein is not frequently expressed on tumor cells

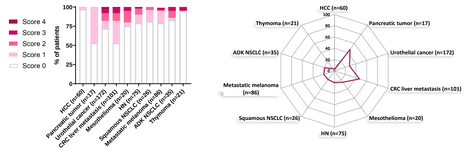
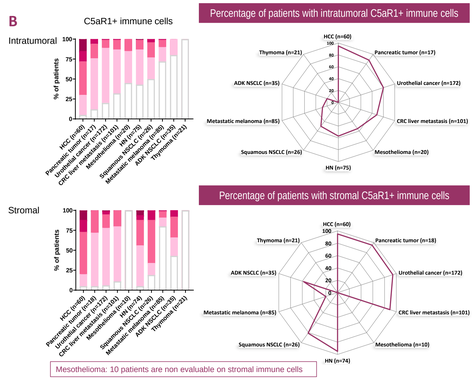
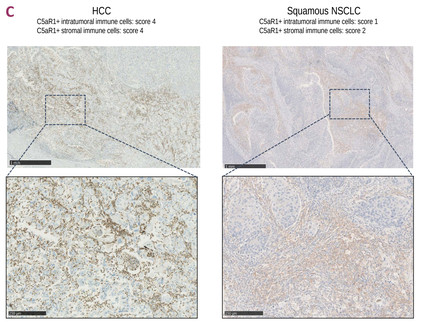


FIGURE 3B: C5aR1 protein may be highly expressed in tumor infiltrating immune cells



Mesothelioma: 10 patients are non evaluable on stromal immune cells

FIGURE 3C: Examples of staining for C5aR1 protein in liver and lung cancers



C5aR immunostaining was performed on a Ventana Benchmark Ultra machine with a mouse IgG1 anti-huC5aR1 (clone S5/1) antibody from Hycult. Staining was assessed by different pathologists according to the indication. The proportions of tumor and immune cells stained were evaluated as follows: Score 0: no positive cells; Score 1: <10% positive cells; Score 2: between 10 and 50% positive cells; Score 4: >80% positive cells

Conclusion

- Anaphylatoxin C5a is released into the cancer microenvironment and binds the C5aR1 receptor, promoting protumoral inflammation and immune suppression through the recruitment and activation of myeloid-derived suppressor cells (MDSC) and neutrophils [3]. We confirm the expression of C5aR1 in several primary tumor types and its correlation with poor prognosis [4-7]. C5aR1 is expressed by TME-infiltrating myeloid cells. IPH5401, a fully human anti-C5aR1 antibody, inhibits C5a-mediated effects on neutrophils *in vitro*.
- Interestingly, C5aR1 is not expressed on tumor-infiltrating DC in lung tumors. C5aR blockade with IPH5401 should therefore act solely on myeloid suppressor cells, without impairing the tumor-specific immune response.
- C5aR1 and PD-L1 mRNA levels are poorly correlated in primary tumors. The combined blockade of C5aR1 and anti-PD-1 synergistically reduces tumor growth and delays tumor progression in mouse preclinical models [8]. By blocking both these pathways, we can induce complementary anti-tumor effects by activating T cells and impeding the immunosuppression mediated by myeloid cells in the tumor microenvironment. These data suggest that IPH5401 can increase anti-PD-(L)1 antibody efficacy and overcome secondary resistance to anti-PD-(L)1 therapies. The STELLAR-001 clinical trial is currently testing the combination of IPH5401 with durvalumab in IO-pretreated NSCLC and IO-naive HCC. It will provide safety and efficacy data and translational analyses of tumor biopsies [1].

References

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