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Abstract
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Combination of IPH5201, a blocking antibody targeting the CD39 immunosuppressive pathway, with durvalumab and chemotherapies: Preclinical rationale

SCAN ME

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Abstract

Background:

CD39 is an extracellular ectonucleotidase highly expressed in the tumor microenvironment (TME) that, sequentially with CD73, contributes to the production of adenosine (Ado), via hydrolysis of adenosine triphosphate (ATP). IPH5201 is a blocking anti-CD39 monoclonal antibody (mAb) that may promote antitumor immunity by accumulating immunostimulatory ATP released by necrotic cells and reducing immunosuppressive Ado levels in the TME. Targeting the Ado pathway has recently been reported to improve Durvalumab efficacy in early-stage Non-Small Cell Lung Cancer (NSCLC) patients, through the use of Oleclumab, an anti-CD73 mAb. In a first-in-human study (NCT04261075), IPH5201 was well tolerated alone or in combination with Durvalumab. Here we explored preclinical efficacy of IPH5201 in combination with anti-PD-L1 and chemotherapies.

Methods:

CD39 expression was assessed in early and late-stage NSCLC biopsies. *In vitro*, chemotherapies were assessed for their ability to induce ATP release by dying tumor cells and IPH5201 was evaluated for its ability to accumulate the released ATP. *In vivo*, human (hu) CD39 Knock-In (KI) mice were used to engraft syngeneic tumors and evaluate the efficacy of a mouse (mo) version of IPH5201 (moIPH5201) in combination with chemotherapies and anti-PD-L1.

Results:

CD39 was expressed in both squamous (sq) and adenocarcinoma (ad) subtypes of NSCLC with expression noted across disease stages. In a mouse tumor model engrafted in huCD39KI mice, moIPH5201 was able to decrease the human CD39 enzymatic activity and to lower the Ado level *in situ*. *In vitro*, chemotherapies induced extracellular ATP release by tumor cells and IPH5201 was able to accumulate the released ATP, following chemotherapy treatment. Finally, *in vivo*, in a mouse tumor model engrafted in huCD39KI mice, moIPH5201 improved the anti-tumor efficacy of gemcitabine and anti-PD-L1 combination.

IPH5201 Mode of Action

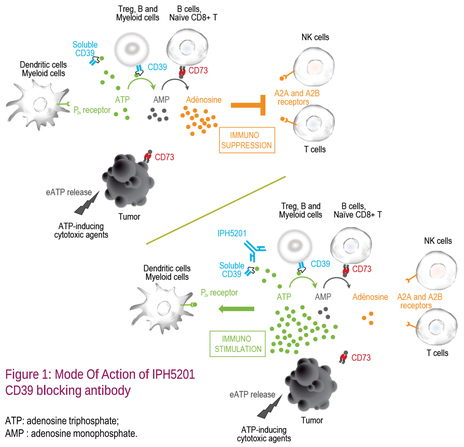


Figure 1: Mode Of Action of IPH5201 CD39 blocking antibody

ATP: adenosine triphosphate;
AMP : adenosine monophosphate.

Chemotherapies induce extracellular ATP release by NSCLC tumor cells

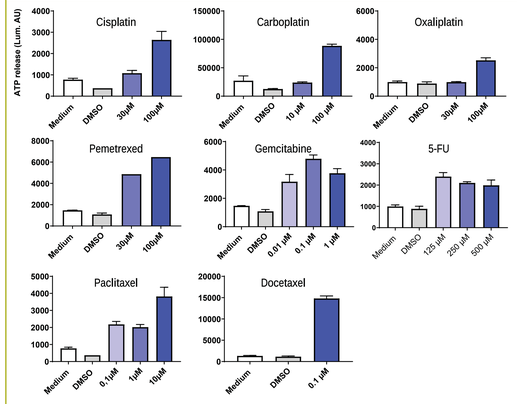


Figure 4: ATP release from H1703 tumor cell line (sqNSCLC) post chemotherapy treatments

Concentrations of chemotherapies inducing some but not complete cell cytotoxicity were selected for the purpose of this cell-based assay. The measure of eATP release in the culture supernatant was performed with a luminescent-based assay (CellTiter-Glo®) between 24h-72h after chemotherapies treatment. eATP release is expressed in Luminescence Arbitrary Unit (AU). Means +/- SD. Of note, equivalent results were obtained with the A549 tumor cell line (adNSCLC).

CD39 is expressed in tumor microenvironment in mouse models and moIPH5201 significantly reduces adenosine levels in tumors *in vivo*

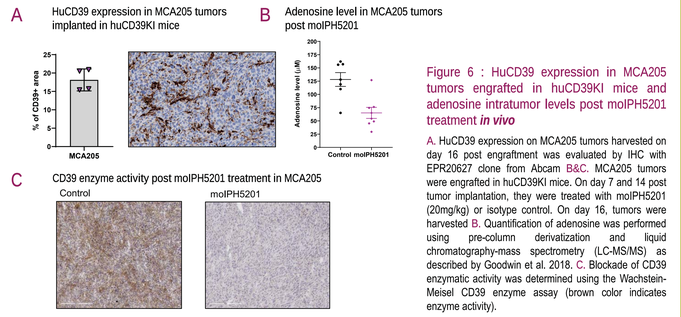


Figure 6 : HuCD39 expression in MCA205 tumors engrafted in huCD39KI mice and adenosine intratumor levels post moIPH5201 treatment *in vivo*

A. HuCD39 expression on MCA205 tumors harvested on day 16 post engraftment was evaluated by IHC with EPR20627 clone from Abcam B&C. MCA205 tumors were engrafted in huCD39KI mice. On day 7 and 14 post tumor implantation, they were treated with moIPH5201 (20mg/kg) or isotype control. On day 16, tumors were harvested B. Quantification of adenosine was performed using pre-column derivatization and liquid chromatography-mass spectrometry (LC-MS/MS) as described by Goodwin et al. 2018. C. Blockade of CD39 enzymatic activity was determined using the Wachstein-Meisel CD39 enzyme assay (brown color indicates enzyme activity).

moIPH5201 improves MC38 tumor control when combined to gemcitabine and anti-PD-L1 treatments

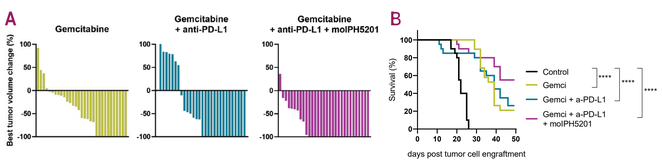


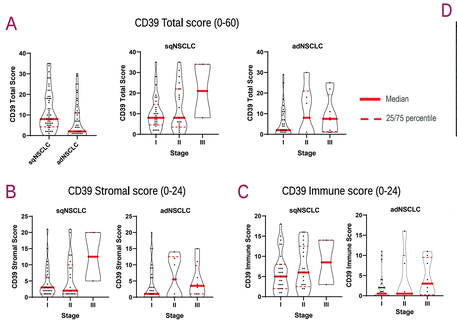
Figure 7 : Tumor growth of MC38 tumor in huCD39KI mice post treatment with gemcitabine +/- anti-PD-L1 +/- moIPH5201

MC38 tumor-bearing huCD39KI mice (n=31) were randomized on day 7 and then treated with 25 mg/kg ip of gemcitabine or PBS, 200 µg ip of anti-mouse PD-L1 Ab or corresponding isotype control Ab and with 400 µg iv of moIPH5201 or corresponding isotype control Ab. A. Graphs show the best tumor volume change in percentage in each treated group in comparison to the control group. B. Mouse survival in each group.

Results

CD39 expression in NSCLC

CD39 is expressed in squamous (sq) and adenocarcinoma (ad) NSCLC, whatever the stage, on stromal and immune cell populations predominantly



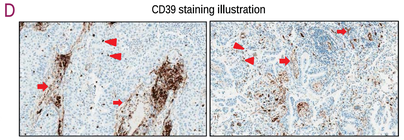


Figure 2 : CD39 expression in NSCLC squamous and adenocarcinoma subtypes

CD39 staining was performed with EPR20627 clone from Abcam on 50 sqNSCLC and 50 adNSCLC FFPE samples. CD39 expression scoring (0-12) is a combination of staining frequency (0-4) and intensity (1-3) A. CD39 total score is the sum of the stromal, immune and tumor expression scores (0-60). B. Stromal score is the sum of the vascular (0-12) and connective tissue (0-12) expression scores. C. Immune score is the sum of the small immune cell (0-12) and large immune cell (0-12) scores. Tumor score (0-12) is not illustrated as poor CD39 staining was observed on tumor cells. D. Red arrows indicate stromal CD39+ cells and red triangles immune CD39+ cells.

IPH5201 blocks ATP degradation

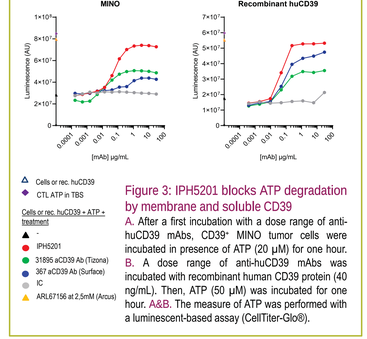


Figure 3: IPH5201 blocks ATP degradation by membrane and soluble CD39

A. After a first incubation with a dose range of anti-huCD39 mAbs, CD39+ MINO tumor cells were incubated in presence of ATP (20 µM) for one hour. B. A dose range of anti-huCD39 mAbs was incubated with recombinant human CD39 protein (40 ng/mL). Then, ATP (50 µM) was incubated for one hour. A&B. The measure of ATP was performed with a luminescent-based assay (CellTiter-Glo®).

IPH5201 blocks CD39-mediated degradation of eATP released after chemotherapy treatment

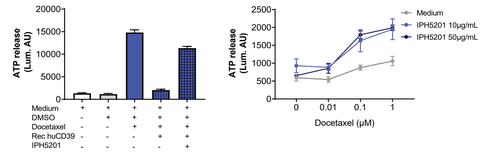


Figure 5: eATP release from CD39 negative (H1703) and CD39 positive (OAW42) tumor cells post docetaxel and IPH5201 treatment

A. H1703 (CD39-) cells were incubated with recombinant huCD39 (400 ng/mL) to mimic soluble CD39 in tumor microenvironment with or without IPH5201 at 10 µg/mL and then treated with docetaxel (0,1µM). The measure of eATP release was performed 40h after docetaxel treatment. B. OAW42 (CD39+) cells were incubated with 10 or 50 µg/mL of IPH5201 and treated with a dose range of docetaxel. The measure of eATP release was performed 30h after docetaxel treatment. It was measured in the cell culture supernatant with a luminescent-based assay (CellTiter-Glo®) and expressed in Luminescence Arbitrary Unit (AU). Means +/- SD.

Conclusion and perspectives

IPH5201 was shown to block CD39 enzymatic activity, to lower Adenosine intratumoral levels and to increase extracellular ATP release by tumor cells upon chemotherapy treatment, and finally to improve anti-tumor efficacy in preclinical models in combination with chemotherapies and PD-L1 blockade. Altogether, the expression profile of CD39 in early-stage NSCLC and preclinical combination data support the clinical evaluation of IPH5201 in combination with Durvalumab and chemotherapies in early-stage NSCLC patients. A Phase II Multicenter, open label, non-randomized study of neoadjuvant and Adjuvant Treatment with IPH5201 and durvalumab in patients with resectable, early-Stage (II to IIIA) Non-Small Cell Lung Cancer (MATISSE) is expected to start in 2023.

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Carine Paturel is an employee and shareholder of Innate Pharma