IPH4301, an antibody targeting MICA and MICB, exhibits potent cytotoxic activity and immunomodulatory properties for the treatment of cancer

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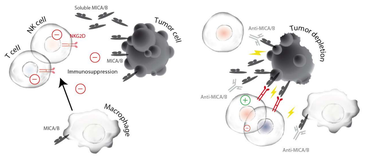
Introduction

MICA and MICB are highly polymorphic ligands for the activating receptor NKG2D expressed on NK cells and CD8+ T cells. NKG2D ligands are not expressed by resting normal cells, but are induced by cellular stress e.g. viral, bacterial infection or tumor transformation. In transformed cells MICA/B can be further upregulated by some chemotherapies, radiotherapy and cytokines. Recently, MICA/B expression was also described on tumor associated, immunosuppressive macrophages. Chronic exposure to membrane-bound or soluble MICA/B downregulates NKG2D.

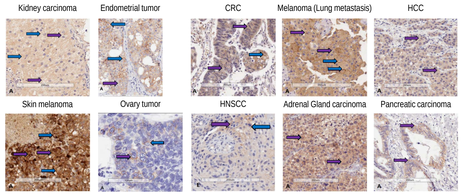
We have generated an anti-MICA/B antibody that neutralizes immunosuppressive MICA/B and induces tumor killing by Antibody-Dependent Cell Cytotoxicity (ADCC).

1. Mechanism of Action



Target expression

2. MICA/B expression is induced in numerous tumor types



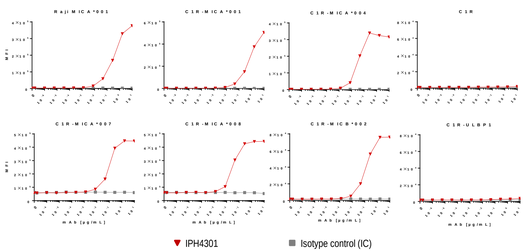
Immunohistochemical detection of MICA/B in formalin-fixed, paraffin-embedded (FFPE) sections of indicated tumor samples. Sections were stained with the anti-MICA/B mAb (clone BAMO1).

3. Prevalence of MICA/B expression across tumor types

Tumor localization	Kidney	Large intestine/Sigmoid colon	Endometrium	Thyroid Gland	Metastatic melanoma	Liver	Breast (TMA)	Melanoma	Lung (TMA)	Ovary	Head & Neck	Intestine/colon (TMA)	Prostate (TMA)	Adrenal Gland	Pancreas
Positive Cases (n=)	100% (20)	100% (20)	100% (10)	100% (10)	90% (10)	85% (20)	82% (28)	71% (7)	62% (29)	60% (10)	59% (17)	50% (32)	50% (8)	40% (10)	33% (18)

Characterization of IPH4301, a first-in-class humanized anti-MICA/B mAb

4. IPH4301 has high affinity and crossreactivity to MICA and MICB allotypes



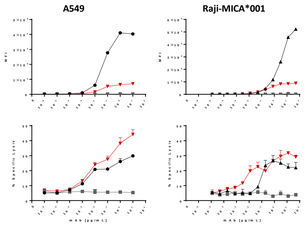
Raji or C1R cell lines transfected with various MICA/B alleles were incubated with IPH4301 (red lines) or IC (grey lines). Primary antibodies were revealed with a PE-conjugated goat anti-human IgG. Cells were analyzed by flow cytometry. Mean of fluorescence intensity (MFI) is shown on graphs.

Monovalent affinities of IPH4301 to representative MICA/B allotypes measured by surface plasmon resonance

	MICA					MICB	
Allele	*001	*004	*007:01	*008	*027	*005:02	*007
KD (nM)	26.3	0.1	0.1	0.4	0.1	47.8	48.5

In vitro cytotoxicity

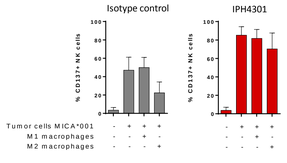
5. IPH4301 mediates potent ADCC by resting primary human NK cells



Binding of indicated mAbs on A549 or Raji-MICA*001 tumor cells was analyzed by flow cytometry. Their respective ADCC efficacy was measured by classical 4h ^{51}Cr release assay using PBMC from healthy volunteers as effector cells (E/T=200/1)

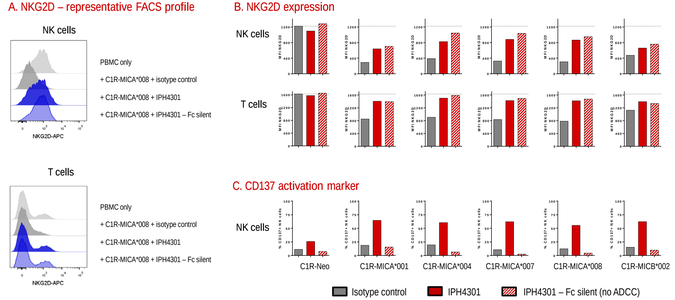
In vitro immunomodulation

6. IPH4301 overcomes M2 macrophages suppression of NK cell activity



NK cells were incubated 24 hours with autologous *in vitro* monocyte-derived M1 or M2 macrophages. Then, culture supernatants and non adherent cells were incubated with LCL-721.221-MICA*001 for an additional 24 hours. Activation marker CD137 on NK was measured by flow cytometry. IPH4301 or IC were used at 10 µg/mL. Mean +/- SD, n=4-7 independent healthy donors.

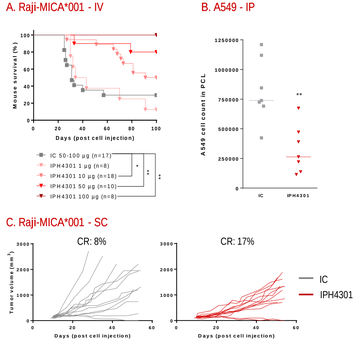
7. IPH4301 blocks MICA/B-induced downmodulation of NKG2D on NK and CD8+ T cells and promotes concomitant NK cell activation



PBMC from healthy volunteers were incubated with indicated tumor cell lines (E/T =5) for 16h at 37°C in presence of 10 µg/mL of indicated antibodies. Cells were analyzed by flow cytometry for NKG2D and CD137 expression on T cells and NK cells.

In vivo efficacy – immunodeficient mouse models

8. IPH4301 shows anti-tumoral *in vivo* efficacy



A: NOD-SCID mice were engrafted i.v. with Raji-MICA*001 cells and treated the same day with a single injection of IPH4301 or IC at indicated doses (µg/mouse, i.v.). Log rank (Mantel-Cox) test, 10 µg p=0.03, 50 µg p=0.008, 100 µg p=0.002.

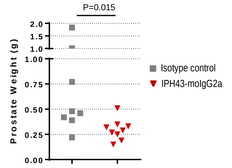
B: NOD-SCID mice (n=7/group) were injected i.p. with A549 cells and treated with a single injection of IPH4301 or IC (10 µg/mouse, i.v.). A549 cell number in peritoneal cavity lavage (PCL) was assessed 24h after treatment. Individual mice and median are represented. Mann-Whitney comparison p=0.002.

C: NOD-SCID mice (n=12/group) were engrafted s.c. with Raji-MICA*001 cells. Mice were randomized at day 10 (tumor volume ~120 mm³) and were then treated with IPH4301 or IC (250 µg/mouse, i.v., twice a week for 3wks). Individual tumor volumes are shown. CR=complete response.

In vivo efficacy – TRAMP/MICB mouse model

9. IPH43-molgG2a cures TRAMP-MICB mice from prostate cancer by restoring histology of prostate gland and inducing NK cell infiltration

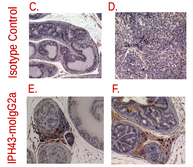
A. Prostate weight



TRAMP/MICB mice are described in Liu et al. Males spontaneously develop prostate tumors expressing human MICB around 20 weeks of age. 22 weeks old males were treated with 300 µg of antibodies (10 mg/kg) twice a week for 7 weeks.

B. Summary of immunohistochemistry analysis

Histology	Isotype control (n=8)		IPH43-molgG2a (n=9)	
Normal/cured	0/8		4/9 (E)	NK infiltration
Well differentiated	5/8 (C)	No NK infiltration	5/9 Localized or stabilized tumor (F)	
Poorly differentiated	3/8 (D)		0/9	



C&D: Mice from control group treated with IC have a similar histology as compared to 29 weeks-old untreated mice.

E&F: IPH43-molgG2a enhanced NK cells infiltration in tumors resulting in tumor elimination or control of tumor progression. E: Over 80% of the prostate glands showed normal histology. F: All the glands showed well-differentiated prostate tumor (localized or stabilized) histopathology.

Conclusion

- We have generated and humanized a pan-allele anti-MICA/B antibody, IPH4301.
- IPH4301 efficiently mediates ADCC towards tumor cells expressing various alleles of MICA or MICB. IPH4301-mediated ADCC overcomes M2 macrophages-induced NK cell immune suppression.
- NKG2D downmodulation by chronic exposure to MICA/B is inhibited by IPH4301 on primary lymphocytes. In addition, IPH4301 efficiently activates NK cells and induces ADCC-mediated tumor cell lysis.
- IPH4301 shows *in vivo* efficacy in immunodeficient mice. IPH43-molgG2a cures and/or normalizes histology of spontaneously arising prostate tumors in TRAMP/MICB immunocompetent mice. IPH43-molgG2a induces NK cell recruitment into MICB positive tumors.
- We have chosen the anti-MICA/B IPH4301 for the development of a therapeutic cytotoxic antibody with immune regulation properties. IND-enabling studies will start in 2016.

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