BYON4228, an antagonistic SIRPα mAb with a unique and favorable preclinical profile compared to three comparator SIRPα mAbs

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BACKGROUND

In preclinical models, the CD47-SIRPα axis has been firmly established as an immune checkpoint that inhibits myeloid-derived antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) mediated by tumor-targeting antibodies, thereby limiting the efficacy of these antibodies. Initial therapeutic strategies targeting the CD47-SIRPα axis focused on CD47 blockers and more recently also SIRPα antagonistic monoclonal antibodies (mAbs) have entered clinical studies. The latter approach is thought to be more advantageous since SIRPα expression is primarily myeloid restricted, whereas CD47 is broadly expressed and, besides SIRPα, binds other ligands as well, such as SIRPγ. We have generated and characterized preclinically the antagonistic SIRPα-targeting mAb BYON4228 and performed head-to-head comparison studies with three mAbs, namely 1H9[1], HEFLB[2], and SIRPAB-11[3], that in all probability represent three clinical stage SIRPα-targeting mAbs. These studies focused on: 1) recognition and functionality of both common allelic forms of SIRPα ($SIRP\alpha_{BIT}$ and $SIRP\alpha_1$); 2) recognition and blocking of SIRPγ on T lymphocytes which is reported to affect T cell extravasation and activation; 3) functionality of the antibody Fc-region which can mediate depletion of SIRPα-positive host myeloid immune effector cells thereby compromising a host defense and undermining the effect of immunotherapy.

LEGENDS FOR ALL FIGURES

- BYON4228 (IgG1-L234A/L235A)
- 1H9 (IgG1-N297A)
- HEFLB (IgG4-S228P/L445P)
- SIRPAB-11 (IgG1-K322A)
- Isotype control (IgG1-L234A/L235A)
- Isotype control (IgG1-N297A)
- Isotype control (IgG4-S228P/L445P)
- Isotype control (IgG1-K322A)

IN VITRO BINDING

BYON4228 is a potent pan-allelic SIRPα binder lacking binding to SIRPγ

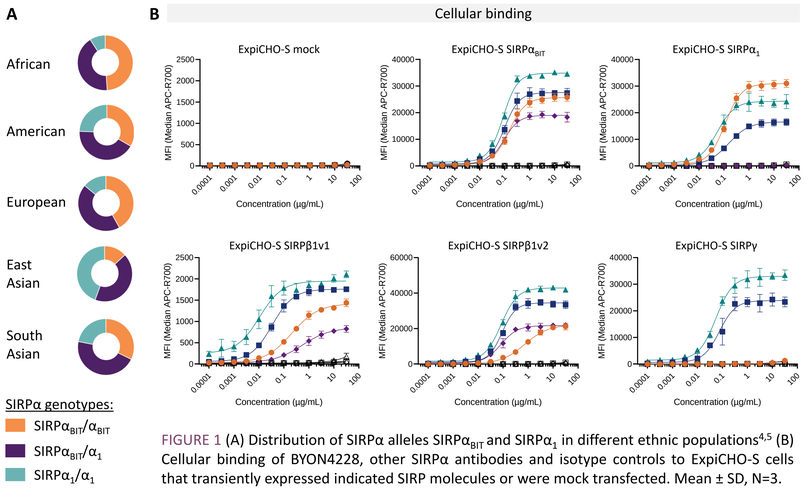


FIGURE 1 (A) Distribution of SIRPα alleles $SIRP\alpha_{BIT}$ and $SIRP\alpha_1$ in different ethnic populations[4,5] (B) Cellular binding of BYON4228, other SIRPα antibodies and isotype controls to ExpiCHO-S cells that transiently expressed indicated SIRP molecules or were mock transfected. Mean ± SD, N=3.

BYON4228 binds to the CD47-binding site of SIRPα in a pan-allelic fashion

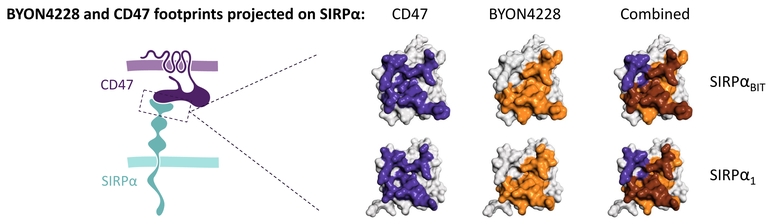


FIGURE 2 CD47 binding site[6] (purple, left), BYON4228 footprint mapped by HDX-MS (orange, middle) and overlap (brown, right) projected onto the N-terminal Ig-like CD47-binding domains of $SIRP\alpha_{BIT}$[7] and $SIRP\alpha_1$[6].

BYON4228 competitively blocks binding of CD47 to SIRPα on myeloid cells

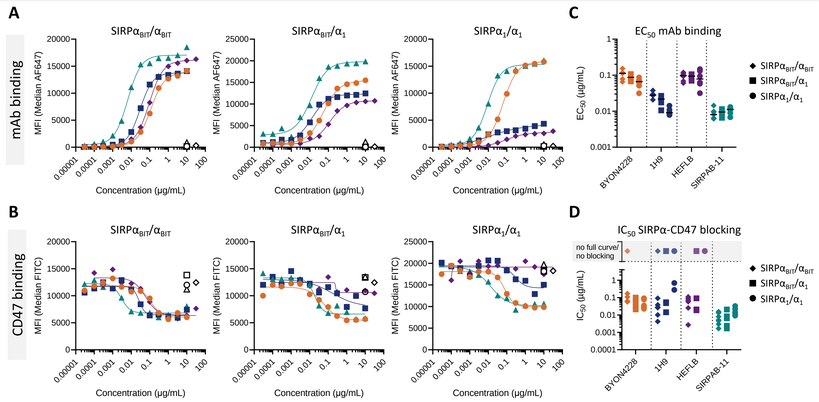


FIGURE 3 Binding of BYON4228 and other anti-SIRPα antibodies to primary monocytes with the indicated SIRPα genotypes (A) and blocking of the binding of CD47 coated fluorescent beads to these cells (B). Three representative donors are shown. Binding EC_{50} (C) and blocking IC_{50} (D) of all mAbs for all tested donors. IC_{50} values could not be calculated from donors with incomplete curve saturation or without any response as indicated in the graph.

BYON4228 does not bind or block T cell-expressed SIRPγ

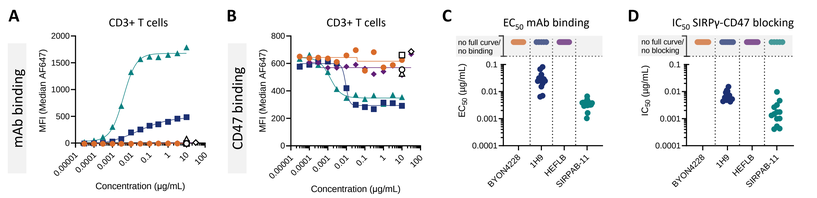


FIGURE 4 BYON4228 does not bind (A) or block the binding of CD47 coated fluorescent beads (B) to primary CD3+ T cells. A representative donor is shown. Binding EC_{50} (C) and blocking IC_{50} (D) of all mAbs for all tested donors. EC_{50} and IC_{50} values could not be calculated from donors with incomplete curve saturation or without any response as indicated in the graph. Four donors that lacked CD47-binding despite SIRPγ expression are shown at the top of the IC_{50} graph as well.

IN VITRO AND *IN VIVO* FUNCTIONAL ACTIVITIES

BYON4228-induced pan-allelic ADCP (mΦ) and ADCC (neutrophils) enhancement

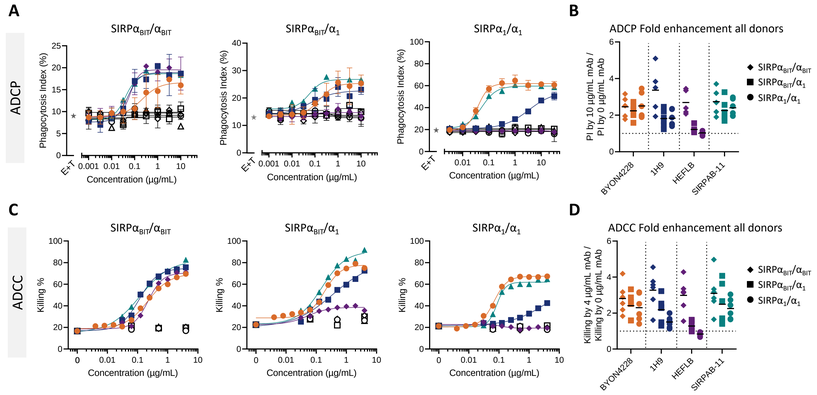


FIGURE 5 Enhancement of macrophage antibody dependent cellular phagocytosis (ADCP) and neutrophil induced antibody dependent cellular cytotoxicity (ADCC) by BYON4228 and other SIRPα antibodies. (A) ADCP of rituximab-opsonized Raji cells in presence of indicated mAbs. Three representative donors are shown. *Phagocytosis index (PI) = number of phagocytosed tumor cells / total number of macrophages * 100.* E+T = effector + target control (rituximab only). (B) ADCP fold enhancement of all tested donors. (C) Neutrophil ADCC of trastuzumab-opsonized SK-BR-3 in presence of indicated mAbs. Three representative donors are shown. (D) ADCC fold enhancement of all tested donors.

BYON4228 does not induce ADCP (mΦ) of SIRPα-expressing cells

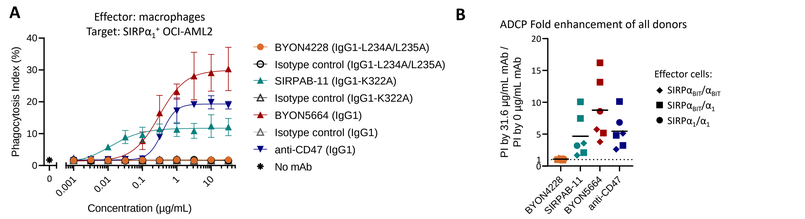


FIGURE 6 BYON4228 does not induce macrophage-mediated phagocytosis of SIRPα-expressing OCI-AML2 cells in contrast to its wildtype-IgG1 equivalent BYON5664, SIRPAB-11 and anti-CD47 (B6H12). (A) Macrophage ADCP of OCI-AML2 in presence of indicated mAbs. Results for a representative donor ($SIRP\alpha_1/\alpha_{BIT}$) are shown. (B) ADCP fold enhancement of all tested donors.

BYON4228 improves the efficacy of rituximab in huSIRPα$_{BIT}$-scid mice *in vivo*

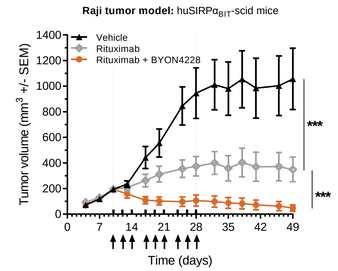


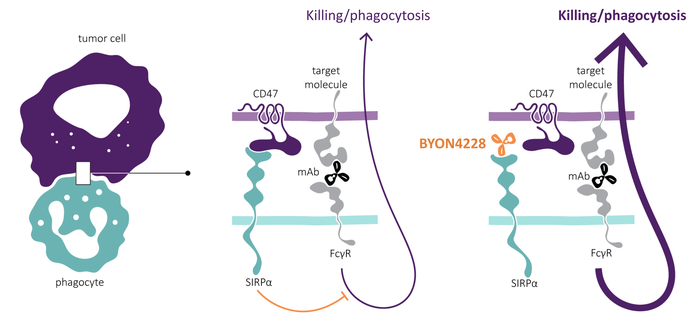
FIGURE 7 Growth curve of Raji cells (subcutaneous) in female huSIRPα$_{BIT}$-scid mice treated IP with vehicle, rituximab monotherapy, or rituximab in combination with BYON4228. The full-length human $SIRP\alpha_{BIT}$ cDNA sequence was inserted into the *Gt(ROSA)26Sor* locus and crossed to Cepba$^{CRE/+}$ mice[8], creating huSIRPα Tg mice with a selective expression on myeloid cells. These mice where then crossed with scid mice to yield huSIRPα$_{BIT}$-scid mice. All compounds were administered IP three times a week (indicated by arrows between dotted lines). Rituximab was used at 1 mg/kg for monotherapy and in combination with 5 mg/kg BYON4228. N=11 per group. Statistical analysis of area under the curve from randomization to end of study; *** p<0.001, Dunnett's multiple comparison test.

CONCLUSIONS

BYON4228 shows:

- **Pan-allelic recognition of SIRPα** in contrast to HEFLB
- **No binding to T cell SIRPγ** in contrast to SIRPAB-11 and 1H9, leaving T cell functions unimpeded[9,10]
- **Blocking of the CD47-SIRPα axis:** it competitively blocks CD47 binding
- ***In vitro* and *in vivo* enhancement of immune cell effector functions** induced by anti-tumor mAbs
- **No depletion of SIRPα-expressing myeloid cells** due to its impaired Fc-region, in contrast to SIRPAB-11
- **Broad potential clinical applicability:** enhancing cellular effector functions in combination with multiple anti-tumor mAbs, incl. trastuzumab, rituximab, daratumumab, cetuximab and panitumumab
- **Unique and favorable profile** compared to three comparator SIRPα mAbs

BYON4228 also showed a favorable safety profile in cynomolgus monkeys and clinical studies will start in 2023 (NCT05737628).



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Online poster:

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