Design, synthesis and biological evaluation of a new Toll Like Receptor 4 agonist-based Antibody Drug Conjugate for cancer immunotherapy.

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TLR4 SIGNALLING IN INFLAMMATION

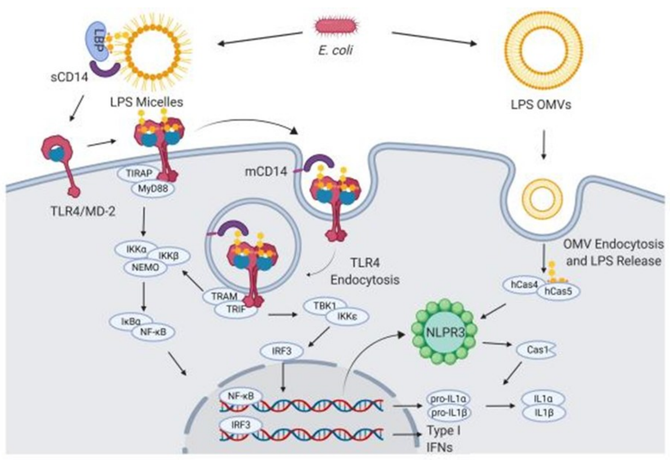


Figure 1:
TLR4 signalling pathway

TLR4 is a transmembrane Pattern Recognition Receptor (PRR), deputed to recognizing highly conserved Damage or Pathogen Associated Molecular Patterns (DAMPs, PAMPs) derived from cellular damage or pathogen infection. Upon activation TLR4 initiates the innate immune response (**Fig. 1**), triggering NF-κB translocation and pro-inflammatory cytokines release.[1,2] The natural ligand of this receptor is lipopolyasccharide (LPS, **Fig. 2**), a PAMP derived from the outer membrane of Gram-negative bacteria. The polysaccharide portion of LPS is divided in O-specific chain (variable among various bacteria classes), outer core (more conserved), and inner core (highly conserved). The lipophilic region is called Lipid A (**Fig. 3**), which is composed by two glucosamine units bearing different lipidic chains (chain length is variable among different bacterial classes), and two phosphates. Lipid A is the minimal part of LPS recognized by TLR4 required to start the immune response, and it's highly toxic.[2,3] Molecules upregulating TLR4 signalling can be exploited as vaccine adjuvants or in immunotherapy, as it is known that cancer elude immune system through TLR4 pathway, shifting macrophage phenotype from M1 to M2.[4]

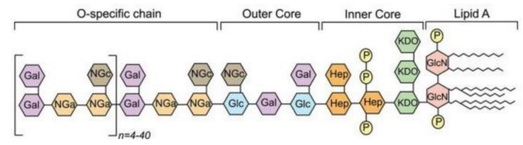


Figure 2:
LPS, the natural ligand of TLR4

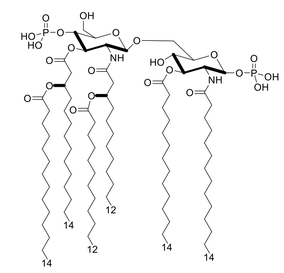


Figure 3:
Structure of Lipid A

LIPID A ANALOGUES AS TLR4 AGONISTS IN IMMUNOTHERAPY

FP20 AND FP20Glyco

Our glicolipidic TLR4 agonists **FP20** and its glycosylated derivatives **FP20Glyco** (Fig. 4) are:

Active on HEK-Blue hTLR4 macrophages in a dose dependent manner (Graph. 1).[5]

Selective on TLR4 over TLR2 (Graph 1 and 2).[5]

Non toxic fot THP-1 derived macrophages (Graph 3).[6]

Active on THP1 macrophages (Graph 4).[6]

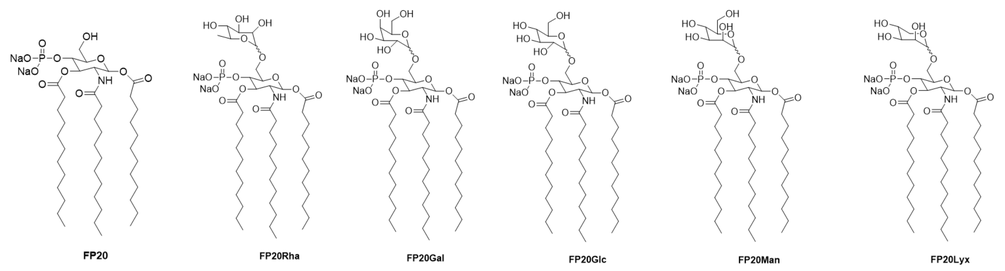
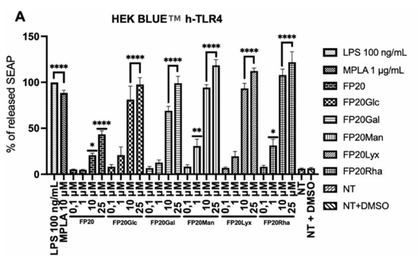
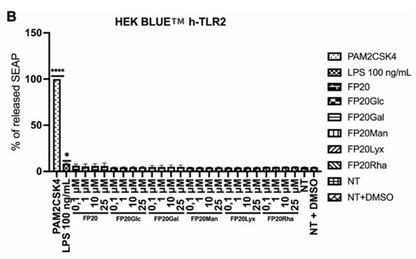


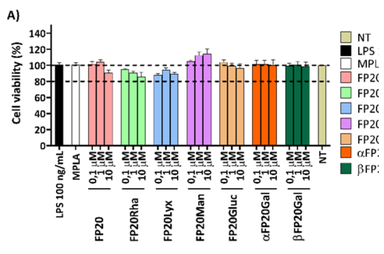
Figure 4:
Lipid A analogues produced by our group



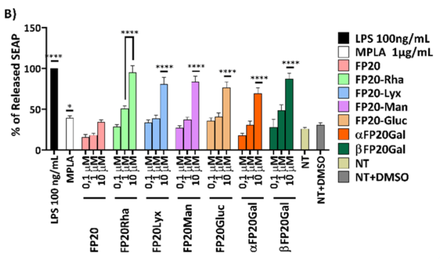
Graphic 1



Graphic 2



Graphic 3

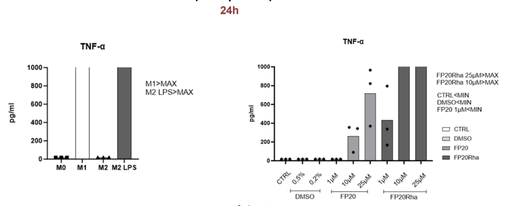


Graphic 4

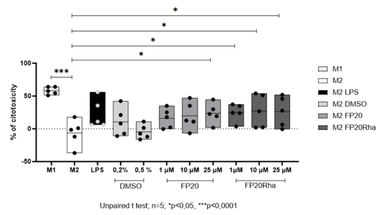
ANTICANCER ACTIVITY

FP20 and **FP20Rha** showed to be able to shift TNF-α production in macrophages to M1 like. Thus switching macrophages phenotype from M2 to anti-cancer M1. (Graph 5)

These macrophages proved to be cytotoxic against leukemia 697 cell line (Graph. 6).



Graphic 5



Graphic 6

FINAL ANTIBODY DRUG CONJUGATE AND SYNTHETIC STRATEGY

AIM OF THE WORK

The biological activity of **FP20** and **FP20Rha** and their ability to shift macrophages' phenotype suggests their use as payloads in an antibody drug conjugate (ADC) for cancer immunotherapy. We will produce a drug-linker system suitable for subsequent bioconjugation with different antibodies. The immunostimulant drug (**FP20** or **FP20Rha**) will be conjugated to the Valine-Citrulline-PABOH linker via phosphate. Val-Cit-PABOH linker is usually used in internalizing ADCs, but recently it has been demonstrated to be exploited for extracellular release.[7] A maleimidocaproyl (MC) moiety will be exploited to obtain bioconjugation with through a Michael addiction with cysteins present on the antibodies (Fig. 5).

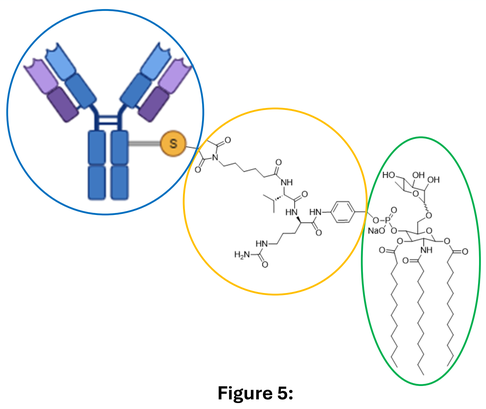


Figure 5:
Final ADC structure

ADC

PAYLOAD

Active drug: usually a cytotoxic chemotherapeutic or an immunostimulant.

In our case **FP20** or **FP20Rha.**

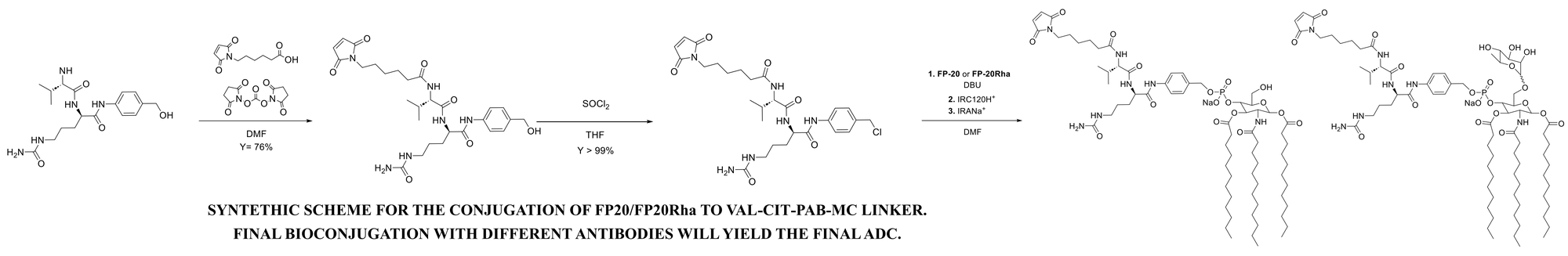
LINKER

Cleaved only in cancer microenvironment by overexpressed proteins.

We selected the Cathepsin cleavable VAL-CIT linker. The self immolative spacer para-amminobenzyl alcohol (PAB) will degrade upon cleaving of the linker, releasing the drug in its active form.

ANTIBODY

The antibody is responsible for the selective drug delivery to cancer regions. Conjugation to different antibodies will consent to target different cancer types.



SYNTETHIC SCHEME FOR THE CONJUGATION OF FP20/FP20Rha TO VAL-CIT-PAB-MC LINKER. FINAL BIOCONJUGATION WITH DIFFERENT ANTIBODIES WILL YIELD THE FINAL ADC.

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