

- 5 BTK is activated upstream by Src-family kinases Blk, Lyn and Fyn and leads to downstream activation of essential cell survival pathways such as NF- $\kappa$ B and MAP kinase. BTK contains a pleckstrin-homology (PH) domain that binds phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P<sub>3</sub>). PI(3,4,5)P<sub>3</sub> binding induces Btk to phosphorylate phospholipase C $\gamma$  (PLC $\gamma$ ), which in turn hydrolyzes PI(4,5)P<sub>2</sub>, a phosphatidylinositol, into two second messengers, inositol triphosphate (IP<sub>3</sub>) and diacylglycerol (DAG), which then go on to modulate the activity of downstream proteins during B-cell signalling. Subsequently, Ca<sup>2+</sup> is mobilized and NF- $\kappa$ B and MAP kinase pathways are activated.
- 10 An exemplary BTK inhibitor described in the art is ibrutinib (PCI-32765; Advani et al.; J Clin Oncol. 2013; Jan 1; 31(1), page 88-94) which is a small molecule irreversible BTK inhibitor.

### **Summary**

- 15 We have now found out that the combination of type I anti-CD20 antibody, or an afucosylated, type II anti-CD20 antibody with a BTK inhibitor showed significantly enhanced antiproliferative effects. Surprisingly, this combination is more than additive, i.e. highly synergistic.

- 20 The disclosure relates to an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, for the treatment of cancer in combination with a BTK inhibitor. One aspect of the disclosure is use of an anti-CD20 antibody selected from a type I anti-CD20 antibody and obinutuzumab, and 6-amino-9-[(3R)-1-(2-butynoyl)-3-pyrrolidinyl]-7-(4-phenoxyphenyl)-7,9-dihydro-8H-purin-8-one or a salt thereof, in the manufacture of a medicament for combination therapy of a CD20 expressing cancer.
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The disclosure relates to the use of an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297,

for the manufacture of a medicament for the treatment of cancer in combination with a BTK inhibitor.

5 The disclosure also relates to a method of treatment of patient suffering from cancer by administering an afucosylated anti-CD20 antibody with an amount of fucose of 60% or less of the total amount of oligosaccharides (sugars) at Asn297, in combination with a BTK inhibitor, to a patient in the need of such treatment.

In one embodiment, the amount of fucose is between 40% and 60% of the total amount of oligosaccharides (sugars) at Asn297. In another embodiment, the amount of fucose is 0% of the total amount of oligosaccharides (sugars) at Asn297.

In one embodiment, the type I anti-CD20 antibody is rituximab. In one embodiment, the afucosylated anti-CD20 antibody is an IgG1 antibody. In another embodiment, said cancer is a CD20 expressing cancer, preferably a lymphoma or lymphocytic leukemia. In one embodiment said afucosylated anti-CD20 antibody is humanized B-Ly1 antibody. In another embodiment, said afucosylated antibody is a type II anti-CD20 antibody.

In one embodiment, said BTK inhibitor is a compound selected from the compounds described in WO2011/152351 and WO2013/081016. Said BTK inhibitor preferably is a compound according to formula I or according to formula I-1 as disclosed herein. Preferably, the BTK inhibitor is 6-amino-9-[(3R)-1-(2-butynoyl)-3-pyrrolidinyl]-7-(4-phenoxyphenyl)-7,9-dihydro-8H-purin-8-one or a salt thereof.

In one embodiment, said type I anti-CD20 antibody is rituximab, said afucosylated anti-CD20 antibody is humanized B-Ly1 antibody and said BTK inhibitor is selected from the group consisting of: the compounds described in WO2011/152351 and WO2013/081016. Said BTK inhibitor preferably is a compound according to formula I or according to formula I-1 as disclosed herein. Preferably, the BTK inhibitor is 6-amino-9-[(3R)-1-(2-butynoyl)-3-pyrrolidinyl]-7-(4-phenoxyphenyl)-7,9-dihydro-8H-purin-8-one or a salt thereof, and said cancer is a CD20 expressing cancer, in one embodiment a lymphoma or lymphocytic leukemia.

In one embodiment, the afucosylated anti-CD20 antibody binds CD20 with an KD of  $10^{-8}$  M to  $10^{-13}$  M.

One embodiment of the disclosure is a pharmaceutical composition comprising a combination of a type I anti-CD20 antibody (in one embodiment rituximab) or an

**Patent Claims**

1. Use of an anti-CD20 antibody selected from a type I anti-CD20 antibody and obinutuzumab, and 6-amino-9-[(3R)-1-(2-butynoyl)-3-pyrrolidinyl]-7-(4-phenoxyphenyl)-7,9-dihydro-8H-purin-8-one or a salt thereof, in the  
5 manufacture of a medicament for combination therapy of a CD20 expressing cancer.
2. The use according to claim 1, characterized in that said CD20 expressing cancer is a lymphoma or lymphocytic leukemia.
3. The use according to claim 1 or 2, characterized in that said type I anti-CD20  
10 antibody is rituximab.
4. The use according to any one of claims 1 to 3, characterized in that one or more additional other cytotoxic, chemotherapeutic or anti-cancer agents, or compounds or ionizing radiation enhancing the effects of such agents are to be administered.