In WO 2014/022758 a single agent anti-PD-L1 and PD-L2 dual binding antibodies and methods of use is reported.

R. Furumai reported FK228 (Depsipeptide) as a natural prodrug that inhibits class I histone deacetylases (Cancer Res. 62 (2002) 4916).

Summary

Herein are reported methods for increasing the transient gene expression yield using production enhancers that have reduced cytotoxicity e.g. compared to the known production enhancer valproic acid.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a (canonical) NFκB activator, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment the (canonical) NFκB activator is selected from the group consisting of TNFalpha, ligands of members of the interleukin-1/Toll-like receptor (IL-1R/TLR) family, e.g. Flagellin, lipopolysaccharide and interleukin-1beta.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising 2M2P or Quisinostat or Romidepsin, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

One aspect as reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a compound selected from the group consisting of trans-2-methyl 2-pentenoic acid, the broad-spectrum HDAC inhibitor Quisinostat, and the subtype-specific HDAC inhibitor Romidepsin, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment 2M2P or Quisinostat or Romidepsin is added of from one hour to 24 hours after the after transfection of the cells to the cultivation. In one embodiment 2M2P or Quisinostat or Romidepsin is added about three hours after the transfection of the cells to the cultivation. Thus, in one embodiment the

cultivating is in a cultivation medium to which 2M2P or Quisinostat or Romidepsin has been added after the transfection.

In one embodiment 2M2P (trans-2-methyl 2-pentenoic acid) is added to a final concentration in the cultivation medium of about 6-7 mM (at least 3 mM).

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising an atypical/non-canonical NFκB activator, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment the non-canonical NFκB activator is selected from the group consisting of EGF, PMA, and betulinic acid.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a subtype-specific HDAC inhibitor, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment the subtype-specific HDAC inhibitor is an HDAC-1 and/or HDAC-2 and/or HDAC-3 inhibitor. In one embodiment the subtype-specific HDAC inhibitor is selected from the group consisting of pimelic diphenylamide 106, Apicidin, and Romidepsin.

In the above methods different functional pathways are modified in order to increase transient gene expression yield. It has now been found that a further increase in transient gene expression yield is possible if production enhancers using different modes of action, i.e. modifying different functional pathways, are combined. The combination of production enhancer modifying the same functional

pathway did not result in a significant further increase in transient gene expression yield.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising two members of the group consisting of canonical and non-canonical NFκB activators, trans-2-methyl 2-pentenoic acid and subtype-specific HDAC inhibitors, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

One aspect as reported herein is a method method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a first and a second compound selected from the group of compounds consisting of trans-2-methyl 2-pentenoic acid, subtype-specific HDAC inhibitors, and canonical and non-canonical NFκB activators, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising a compound selected from the group consisting of trans-2-methyl 2-pentenoic acid and HDAC inhibitors chemically distinct from short chain fatty acids derivatives and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising Flagellin and trans-2-methyl 2-pentenoic acid, and
- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising Quisinostat and trans-2-methyl 2-pentenoic acid, and

- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

As reported herein is a method for the recombinant production of a polypeptide in a eukaryotic cell comprising the steps of:

- cultivating a eukaryotic cell comprising a nucleic acid encoding the polypeptide in a cultivation medium comprising Romidepsin and one of trans-2-methyl 2-pentenoic acid and Flagellin, and

- recovering the polypeptide from the cell or the cultivation medium and thereby producing the polypeptide in a eukaryotic cell.

In one embodiment 2M2P is added to a final concentration in the cultivation medium of about 6-7 mM (about 3 hours after transfection).

In one embodiment Flagellin or Romidepsin are added at a concentration of 2 ng/mL and 15 nM, respectively, 27 h after transfection.

In one embodiment the eukaryotic cell is a mammalian cell. In one embodiment the mammalian cell is a CHO cell or a HEK cell. In one preferred embodiment the mammalian cell is a HEK293 cell.

In one embodiment the expression is a stable or a transient expression. In one preferred embodiment the expression is a transient expression.

In one embodiment the cultivation is for 3 to 16 days. In one embodiment the cultivation is for 3-10 days, preferably if the cell is a HEK cell. In one preferred embodiment the cultivation is for about 7 days.

Description of the Figures

Figure 1: Relative cell specific productivity of HEK293 cells treated with various VPA concentrations; N = 2 for each treatment; y-axis: relative cell specific productivity [%]; 1: control, 2: 0.5 mM