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(54) Titre : TRAITEMENT DE TUMEURS PAR UNE ASSOCIATION D'UN ADENOVIRUS ONCOLYTIQUE, D'UN INHIBITEUR DE CDK4/6 ET D'UN AUTRE AGENT THERAPEUTIQUEMENT ACTIF

(54) Title: TREATMENT OF TUMORS BY A COMBINATION OF AN ONCOLYTIC ADENOVIRUS, A CDK4/6 INHIBITOR AND A FURTHER THERAPEUTICALLY ACTIVE AGENT

(57) Abrégé/Abstract:
The present invention is related to a combination of an adenovirus, a CDK4/inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor, nutlin or a derivative of nutlin.

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(54) Title: TREATMENT OF TUMORS BY A COMBINATION OF AN ONCOLYTIC ADENOVIRUS, A CDK4/6 INHIBITOR AND A FURTHER THERAPEUTICALLY ACTIVE AGENT

(57) Abstract: The present invention is related to a combination of an adenovirus, a CDK4/inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor, nutlin or a derivative of nutlin.

DEMANDE OU BREVET VOLUMINEUX

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Treatment of tumors by a combination of an oncolytic adenovirus, a CDK4/6 inhibitor and a further therapeutically active agent

The present invention is related to combination of an oncolytic virus and a CDK4/inhibitor; the use of such combination in the treatment of a disease such as tumor; an oncolytic virus, preferably an oncolytic adenovirus for use in the treatment of a disease such as tumor together with a CDK4/6 inhibitor; and a CDK4/6 inhibitor for use in the treatment of a disease such as tumor together with an oncolytic virus, preferably an oncolytic adenovirus.

A number of therapeutic concepts are currently used in the treatment of tumors. Apart from using surgery, chemotherapy and radiotherapy are predominant. All these techniques are, however, associated with considerable side effects. The use of replication selective oncolytic viruses provides for a new platform for the treatment of tumors. In connection therewith a selective intratumor replication of a viral agent is initiated which results in virus replication, lysis of the infected tumor cell and spreading of the virus to adjacent tumor cells. As the replication capabilities of the virus is limited to tumor cells, normal tissue is spared from replication and thus from lysis by the virus.

The problem underlying the present invention is the provision of means so as to increase the efficacy of tumor therapy based on oncolytic viruses and adenovirus in particular.

These and other problems are solved by the subject matter of the attached independent claims; preferred embodiments may be taken from the attached dependent claims.

The problem underling present invention is also solved in a first aspect, which is also a first embodiment of such first aspect by a combination comprising an adenovirus and a CDK4/6 inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor and nutlin or a nutlin derivative.

In the following, further embodiments of such first aspect are disclosed.

Embodiment 2: The combination of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The combination of any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The combination of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The combination of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The combination of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The combination of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The combination of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The combination of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The combination of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The combination of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The combination of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The combination of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The combination of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The combination of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The combination of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The combination of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The combination of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The combination of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The combination of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The combination of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The combination of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The combination of Embodiment 21, wherein the adenovirus is dl520, wherein the adenoviral E3 region is functionally inactive.

Embodiment 24: The combination of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The combination of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The combination of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The combination of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The combination of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The combination of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The combination of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The combination of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The combination of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The combination of any one of Embodiments 1 to 32, wherein the composition further comprises a PARP inhibitor.

Embodiment 34: The combination of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The combination of any one of Embodiments 1 to 32, wherein the composition further comprises a bromodomain inhibitor.

Embodiment 36: The combination of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The combination of any one of Embodiments 1 to 36, wherein the composition further comprises a nutlin or a derivative thereof.

Embodiment 38: The composition of Embodiment 37, wherein the nutlin is nutlin-3.

Embodiment 39: The composition of any one of Embodiments 37 and 38, wherein the derivative of nutlin is selected from the group comprising NVP-HDM201, Idasanutlin, AM-8553, SAR405838, Nutlin-3a, AMG232.

Embodiment 40: The combination of any one of embodiments 1 to 39, wherein the constituents of the combination are for separate administration.

Embodiment 41: The combination of any one of embodiments 1 to 39, wherein the constituents of the combination are for combined administration.

The problem underling present invention is also solved in a second aspect, which is also a first embodiment of such second aspect by the combination according to the first aspect, including any embodiments thereof, for use in the treatment of a diseases, more preferably a tumor or cancer. comprising an adenovirus and a CDK4/6 inhibitor.

In the following, further embodiments of such second aspect are disclosed.

Embodiment 1: A combination comprising an adenovirus and a CDK4/6 inhibitor for use in a method for the treatment and/or prevention of a disease, preferably a tumor or cancer.

Embodiment 2: The combination for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The combination of for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The combination for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The combination for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The combination for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The combination for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The combination for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The combination for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The combination for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The combination for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The combination for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The combination for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The combination for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The combination for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The combination for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The combination for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The combination for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The combination for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The combination for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The combination for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The combination for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The combination for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The combination for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The combination for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The combination for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The combination for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The combination for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The combination for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The combination for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The combination for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The combination for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The combination for use of any one of Embodiments 1 to 32, wherein the composition further comprises a PARP inhibitor.

Embodiment 34: The combination for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The combination for use of any one of Embodiments 1 to 32, wherein the composition further comprises a bromodomain inhibitor.

Embodiment 36: The combination for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The combination for use of any one of embodiments 1 to 36, wherein the constituents of the combination are for separate administration.

Embodiment 38: The combination for use of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The combination for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The combination for use of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The combination for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The combination for use of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The combination for use of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44. The combination for use of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45. The combination for use of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The combination for use of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The combination for use of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The combination for use of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The combination for use of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The combination for use of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The combination for use of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The combination for use of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The combination for use of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The combination for use of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The combination for use of any one of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

The problem underling present invention is also solved in a third aspect, which is also a first embodiment of such third aspect by an adenovirus for use in the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus and a CDK4/6 inhibitor.

In the following, further embodiments of such third aspect are disclosed.

Embodiment 2: The adenovirus for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The adenovirus of for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The adenovirus for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The adenovirus for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The adenovirus for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The adenovirus for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The adenovirus for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The adenovirus for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The adenovirus for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The adenovirus for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The adenovirus for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The adenovirus for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The adenovirus for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The adenovirus for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The adenovirus for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The adenovirus for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The adenovirus for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The adenovirus for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The adenovirus for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The adenovirus for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The adenovirus for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The adenovirus for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The adenovirus for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The adenovirus for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The adenovirus for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The adenovirus for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The adenovirus for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The adenovirus for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The adenovirus for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The adenovirus for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The adenovirus for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The adenovirus for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The adenovirus for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The adenovirus for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The adenovirus for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The adenovirus for use of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor and/or the bromodomain inhibitor are administered to the subject separately or as any combination.

Embodiment 38: The adenovirus for use of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The adenovirus for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The adenovirus for use of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The adenovirus for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The adenovirus for use of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The adenovirus for use of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44. The adenovirus for use of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45. The adenovirus for use of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The adenovirus for use of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The adenovirus for use of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The adenovirus for use of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The adenovirus for use of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The adenovirus for use of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The adenovirus for use of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The adenovirus for use of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The adenovirus for use of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The adenovirus for use of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The adenovirus for use of any one of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

The problem underling present invention is also solved in a fourth aspect, which is also a first embodiment of such fourth aspect by a CDK4/6 inhibitor for use in the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus and a CDK4/6 inhibitor.

In the following, further embodiments of such fourth aspect are disclosed.

Embodiment 2: The CDK4/6 inhibitor for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The CDK4/6 inhibitor of for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The CDK4/6 inhibitor for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The CDK4/6 inhibitor for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The CDK4/6 inhibitor for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The CDK4/6 inhibitor for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The CDK4/6 inhibitor for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The CDK4/6 inhibitor for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The CDK4/6 inhibitor for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The CDK4/6 inhibitor for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The CDK4/6 inhibitor for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The CDK4/6 inhibitor for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The CDK4/6 inhibitor for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The CDK4/6 inhibitor for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The CDK4/6 inhibitor for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The CDK4/6 inhibitor for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The CDK4/6 inhibitor for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The CDK4/6 inhibitor for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The CDK4/6 inhibitor for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The CDK4/6 inhibitor for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The CDK4/6 inhibitor for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The CDK4/6 inhibitor for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The CDK4/6 inhibitor for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The CDK4/6 inhibitor for use of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor and/or the bromodomain inhibitor are administered to the subject separately or as any combination.

Embodiment 38: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The CDK4/6 inhibitor for use of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The CDK4/6 inhibitor for use of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44. The CDK4/6 inhibitor for use of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45. The CDK4/6 inhibitor for use of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The CDK4/6 inhibitor for use of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The CDK4/6 inhibitor for use of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The CDK4/6 inhibitor for use of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The CDK4/6 inhibitor for use of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The CDK4/6 inhibitor for use of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The CDK4/6 inhibitor for use of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The CDK4/6 inhibitor for use of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The CDK4/6 inhibitor for use of any one of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

The problem underling present invention is also solved in a fifth aspect, which is also a first embodiment of such fifth aspect by a PARP inhibitor for use in the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus, a CDK4/6 inhibitor and a PARP inhibitor.

In the following, further embodiments of such fifth aspect are disclosed.

Embodiment 2: The PARP inhibitor for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The PARP inhibitor of for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The PARP inhibitor for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The PARP inhibitor for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The PARP inhibitor for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The PARP inhibitor for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The PARP inhibitor for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The PARP inhibitor for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The PARP inhibitor for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The PARP inhibitor for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The PARP inhibitor for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The PARP inhibitor for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The PARP inhibitor for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The PARP inhibitor for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The PARP inhibitor for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The PARP inhibitor for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The PARP inhibitor for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The PARP inhibitor for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The PARP inhibitor for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The PARP inhibitor for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The PARP inhibitor for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The PARP inhibitor for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The PARP inhibitor for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The PARP inhibitor for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The PARP inhibitor for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The PARP inhibitor for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The PARP inhibitor for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The PARP inhibitor for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The PARP inhibitor for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The PARP inhibitor for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The PARP inhibitor for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The PARP inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The PARP for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The PARP inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The PARP inhibitor for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The PARP inhibitor for use of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor and/or the bromodomain inhibitor are administered to the subject separately or as any combination.

Embodiment 38: The PARP inhibitor for use of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The PARP inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The PARP inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The PARP inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The PARP inhibitor for use of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The PARP inhibitor for use of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44. The PARP inhibitor for use of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45. The PARP inhibitor for use of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The PARP inhibitor for use of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The PARP inhibitor for use of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The PARP inhibitor for use of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The PARP inhibitor for use of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The PARP inhibitor for use of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The PARP inhibitor for use of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The PARP inhibitor for use of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The PARP inhibitor for use of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The PARP inhibitor for use of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The PARP inhibitor for use of any one of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

The problem underling present invention is solved in a sixth aspect, which is also a first embodiment of such sixth aspect by a bromodomain inhibitor for use in the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus, a CDK4/6 inhibitor and a bromodomain inhibitor.

In the following, further embodiments of such sixth aspect are disclosed.

Embodiment 2: The bromodomain inhibitor for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The bromodomain inhibitor of for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The bromodomain inhibitor for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The bromodomain inhibitor for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The bromodomain inhibitor for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The bromodomain inhibitor for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The bromodomain inhibitor for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The bromodomain inhibitor for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The bromodomain inhibitor for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The bromodomain inhibitor for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The bromodomain inhibitor for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The bromodomain inhibitor for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The bromodomain inhibitor for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The bromodomain inhibitor for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The bromodomain inhibitor for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The bromodomain inhibitor for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The bromodomain inhibitor for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The bromodomain inhibitor for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The bromodomain inhibitor for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The bromodomain inhibitor for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The bromodomain inhibitor for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The bromodomain inhibitor for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The bromodomain inhibitor for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The bromodomain inhibitor for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The bromodomain inhibitor for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The bromodomain inhibitor for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The bromodomain inhibitor for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The bromodomain inhibitor for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The bromodomain inhibitor for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The bromodomain inhibitor for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The bromodomain inhibitor for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The bromodomain inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The bromodomain for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The bromodomain inhibitor for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The bromodomain inhibitor for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The bromodomain inhibitor for use of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor and/or the bromodomain inhibitor are administered to the subject separately or as any combination.

Embodiment 38: The bromodomain inhibitor for use of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The bromodomain inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The bromodomain inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The bromodomain inhibitor for use of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The bromodomain inhibitor for use of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The bromodomain inhibitor for use of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44. The bromodomain inhibitor for use of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45. The bromodomain inhibitor for use of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The bromodomain inhibitor for use of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The bromodomain inhibitor for use of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The bromodomain inhibitor for use of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The bromodomain inhibitor for use of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The bromodomain inhibitor for use of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The bromodomain inhibitor for use of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The bromodomain inhibitor for use of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The bromodomain inhibitor for use of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The bromodomain inhibitor for use of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The bromodomain inhibitor for use of any one of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

The problem underling present invention is solved in a seventh aspect, which is also a first embodiment of such sixth aspect by nutlin or a nutlin derivative for use in the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus, a CDK4/6 inhibitor and a bromodomain inhibitor.

In the following, further embodiments of such sixth aspect are disclosed.

Embodiment 2: The nutlin or a nutlin derivative for use of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The nutlin or a nutlin derivative for use any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The nutlin or a nutlin derivative for use of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The nutlin or a nutlin derivative for use of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The nutlin or a nutlin derivative for use of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The nutlin or a nutlin derivative for use of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The nutlin or a nutlin derivative for use of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The nutlin or a nutlin derivative for use of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The nutlin or a nutlin derivative for use of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The nutlin or a nutlin derivative for use of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The nutlin or a nutlin derivative for use of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The nutlin or a nutlin derivative for use of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The nutlin or a nutlin derivative for use of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The nutlin or a nutlin derivative for use of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The nutlin or a nutlin derivative for use of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The nutlin or a nutlin derivative for use of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The nutlin or a nutlin derivative for use of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The nutlin or a nutlin derivative for use of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The nutlin or a nutlin derivative for use of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The nutlin or a nutlin derivative for use of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The nutlin or a nutlin derivative for use of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib, Talazoparib and BMN673.

Embodiment 35: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The nutlin or a nutlin derivative for use of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The nutlin or a nutlin derivative for use of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor, the bromodomain inhibitor and or the nutlin or nutlin derivative are administered to the subject separately or as any combination.

Embodiment 38: The nutlin or a nutlin derivative for use of any one of claims 1 to 37, wherein the nutlin derivative is selected form the group comprising NVP-HDM201, Idasanutlin, AM-8553, SAR405838, Nutlin-3a and AMG232.

Embodiment 39: The nutlin or a nutlin derivative for use of any one of claims 1 to 37, wherein the nutlin derivative is different from Nutlin-3a.

Embodiment 40: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 39, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 41: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 40, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 42: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 40, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 43: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 40, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 44: The nutlin or a nutlin derivative for use of Embodiment 43, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 45: The nutlin or a nutlin derivative for use of Embodiment 43, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 46. The nutlin or a nutlin derivative for use of Embodiment 45, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 47. The nutlin or a nutlin derivative for use of any one of Embodiment 1 to 46, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 48: The nutlin or a nutlin derivative for use of Embodiment 47, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 49: The nutlin or a nutlin derivative for use of claim 48, wherein the resistance is mediated by an ABC transporter.

Embodiment 50: The nutlin or a nutlin derivative for use of claim 49, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 51: The nutlin or a nutlin derivative for use of any one of embodiments 47 to 50, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 52: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 51, wherein the cells of the tumor are Rb-negative.

Embodiment 53: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 52, wherein the cells of the tumor are Rb-positive.

Embodiment 54: The nutlin or a nutlin derivative for use of any one of Embodiments 1 to 53, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 55: The nutlin or a nutlin derivative or for use of any one of Embodiments 1 to 54, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 56: The nutlin or a nutlin derivative for use of Embodiment 55, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 57: The bromodomain inhibitor for use of Embodiment 56, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

The problem underlying the present invention is solved in an eighth aspect, which is also a first embodiment of such seventh aspect, by a combination therapy, wherein such combination therapy comprises administration to a subject in need thereof, of, among others,

a) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined herein according to the fourth aspect, including any embodiment thereof, and a PARP inhibitor, in particular one as defined herein according to the fourth aspect;
b) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined herein according to the fourth aspect, including any embodiment thereof, and a bromodomain inhibitor, in particular one as defined herein according to the fifth aspect;
c) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined herein according to the fourth aspect, including any embodiment thereof, and a nutlin or a nutlin derivative, in particular one as defined herein according to the sixth aspect;
d) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined

herein according to the fourth aspect, including any embodiment thereof, a bromodomain inhibitor, a bromodomain inhibitor, in particular one as defined herein according to the fifth aspect; and a nutlin or a nutlin derivative, in particular one as defined herein according to the sixth aspect;

e) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined herein according to the fourth aspect, including any embodiment thereof, a bromodomain inhibitor, a bromodomain inhibitor, in particular one as defined herein according to the fifth aspect; and a PARP inhibitor, in particular one as defined herein according to the fifth aspect; and

f) an adenovirus, in particular one as defined herein according to the third aspect, including any embodiment thereof; a CDK4/6 inhibitor, in particular one as defined herein according to the fourth aspect, including any embodiment thereof, a bromodomain inhibitor, a bromodomain inhibitor, in particular one as defined herein according to the fifth aspect; a PARP inhibitor, in particular one as defined herein according to the fifth aspect; and and a nutlin or a nutlin derivative, in particular one as defined herein according to the sixth aspect.

It is within the present invention that the various embodiments described in connection with the third aspect, including any embodiment thereof, the fourth aspect, including any embodiment thereof, the fifth aspect, including any embodiment thereof, and the sixth aspect, including any embodiment thereof, can be embodiments of a combination therapy according to the eighth aspect in its various forms, in particular as defined above as a), b), c), d), e) and f).

The problem underling present invention is solved in a ninth aspect, which is also a first embodiment of such seventh aspect by a method for the treatment and/or prevention of a diseases in a subject, more preferably a tumor or cancer, wherein the method comprises administering to the subject an adenovirus and a CDK4/6 inhibitor.

In the following, further embodiments of such ninth aspect are disclosed.

Embodiment 2: The method of Embodiment 1, wherein the adenovirus is an oncolytic adenovirus.

Embodiment 3: The method of any one of Embodiments 1 and 2, wherein the adenovirus is replicating in a YB-1 dependent manner.

Embodiment 4: The method of Embodiment 3, wherein the adenovirus is replication deficient in cells which lack YB-1 in the nucleus, but is replicating in cells which have YB-1 in the nucleus.

Embodiment 5: The method of any one of Embodiments 2 to 4, wherein the adenovirus encodes an oncogene protein, wherein the oncogene protein transactivates at least one adenoviral gene, whereby the adenoviral gene is selected from the group comprising E1B55kDa, E4orf6, E4orf3 and E3ADP.

Embodiment 6: The method of Embodiment 5, wherein the oncogene protein is E1A protein.

Embodiment 7: The method of Embodiment 6, wherein the E1A protein is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 8: The method of Embodiment 6, wherein the E1A protein is incapable of binding a functional Rb tumor suppressor gene product.

Embodiment 9: The method of any one of Embodiments 6 to 8, wherein the E1A protein does not induce the localization of YB-1 into the nucleus.

Embodiment 10: The method of any one of Embodiments 5 to 9, wherein the oncogene protein exhibits one or several mutations or deletions compared to the wildtype oncogene protein E1A.

Embodiment 11: The method of Embodiment 10, wherein the deletion is one selected from the group comprising deletions of the CR3 stretches and deletions of the N-terminus and deletions of the C-terminus.

Embodiment 12: The method of any one of Embodiments 6 to 11, wherein the E1A protein is capable of binding to Rb.

Embodiment 13: The method of any one of Embodiments 6 to 12, wherein the E1A protein comprises one or several mutations or deletions compared to the wildtype oncogene protein, whereby the deletion is preferably a deletion in the CR1 region and/or CR2 region.

Embodiment 14: The method of Embodiment 13, wherein the E1A protein is incapable of binding to Rb.

Embodiment 15: The method of any one of Embodiments 1 to 14, wherein the virus is an adenovirus expressing E1A12 S protein.

Embodiment 16: The method of any one of Embodiments 1 to 15, wherein the virus is an adenovirus lacking expression of E1A13S protein.

Embodiment 17: The method of any one of Embodiments 1 to 16, wherein the virus is an adenovirus lacking a functionally active adenoviral E3 region.

Embodiment 18: The method of any one of Embodiments 1 to17, wherein the virus is an adenovirus lacking expression of E1B 19 kDa protein.

Embodiment 19: The method of any one of Embodiments 1 to 18, wherein the virus is an adenovirus expressing an RGD motif at a fibre.

Embodiment 20: The method of any one of Embodiments 1 to 19, wherein the virus is an adenovirus serotype 5.

Embodiment 21: The method of any one of Embodiment 1 to 20, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Embodiment 22: The method of Embodiment 21, wherein the adenovirus is XVir-N-31.

Embodiment 23: The method of Embodiment 21, wherein the adenovirus is dl520, wherein the adenovirual E3 region is functionally inactive.

Embodiment 24: The method of any one of Embodiment 21 to 23, wherein the adenovirus is dl520, wherein dl520 is lacking expression of E1B 19 kDa protein.

Embodiment 25: The method of any one of Embodiments 21 to 24, wherein the adenovirus is dl520 expressing an RGD motif at a fibre.

Embodiment 26: The method of any one of Embodiments 1 to 25, wherein the virus encodes YB-1.

Embodiment 27: The method of Embodiment26, wherein the gene coding for YB-1 is under the control of a tissue-specific promoter, tumor-specific promoter and/or a YB-1 dependent promoter.

Embodiment 28: The method of Embodiment 27, wherein the YB-1 dependent promoter is the adenoviral E2 late promoter.

Embodiment 29: The method of any one of Embodiments 1 to 28, wherein the CDK4/6 inhibitor is a compound which reduces Rb phosphorylation in a cell, preferably a tumor cell.

Embodiment 30: The method of any one of Embodiments 1 to 29, wherein the CDK4/6 inhibitor is a compound which reduces Rb expression in a cell, preferably a tumor cell.

Embodiment 31: The method of any one of Embodiments 1 to 30, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

Embodiment 32: The method of any one of Embodiments 1 to 31, wherein the CDK4/6 inhibitor causes G1 arrest in a cell and inhibits E2F1.

Embodiment 33: The method of any one of Embodiments 1 to 32, wherein the method further comprises administering a PARP inhibitor to the subject.

Embodiment 34: The method of Embodiment 33, wherein the PARP inhibitor is selected from the group comprising olaparib, veliparib, rucaparib and BMN673.

Embodiment 35: The method of any one of Embodiments 1 to 32, wherein the method further comprises administering a bromodomain inhibitor to the subject.

Embodiment 36: The method of Embodiment 35, wherein the bromodomain inhibitor is selected from the group comprising JQ-1, OTX-015, I-BET151, CPI-0610, I-BET762, CPI203, PFI-1 and MS 436.

Embodiment 37: The method of any one of embodiments 1 to 36, wherein the adenovirus, the CDK4/6 inhibitor, the PARP inhibitor and/or the bromodomain inhibitor are administered to the subject separately or as any combination.

Embodiment 38: The method of any one of Embodiments 1 to 37, wherein cells of the tumor have a disruption of the CDK4/6 signaling pathway.

Embodiment 39: The method of any one of Embodiments 1 to 38, wherein cells of the tumor have an uncontrolled G1-S transition of the cell cycle.

Embodiment 40: The method of any one of Embodiments 1 to 38, wherein cells of the tumor have a loss of function mutation or a deletion in a gene selected from the group comprising RB1 gene, CDKN2A gene and CDKN2B gene.

Embodiment 41: The method of any one of Embodiments 1 to 38, wherein cells of the tumor have an amplification of a gene and/or an activating mutation in a gene.

Embodiment 42: The method of Embodiment 41, wherein the gene is selected from the group comprising CCND1, E2F1, E2F2, E2F3, CDK4 and CDK6.

Embodiment 43: The method of Embodiment 41, wherein the gene is one coding for a component of a mitogenic signaling pathway.

Embodiment 44: The method of Embodiment 43, wherein the mitogenic signaling pathway is selected from the group comprising the PI3K pathway and the MAPK pathway.

Embodiment 45: The method of any one of Embodiment 1 to 44, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

Embodiment 46: The method of Embodiment 45, wherein the pharmaceutically active agent is a cytostatic.

Embodiment 47: The method of claim 46, wherein the resistance is mediated by an ABC transporter.

Embodiment 48: The method of claim 47, wherein the ABC transporter is selected from the group comprising MRP and MDR, in particular MDR-1.

Embodiment 49: The method of any one of embodiments 45 to 48, wherein the resistance is a multiple resistance or polyresistance, particular a multiple or polyresistance against a cytostatic and/or radiation.

Embodiment 50: The method of any one of Embodiments 1 to 49, wherein the cells of the tumor are Rb-positive.

Embodiment 51: The method of any one of Embodiments 1 to 50, wherein the cells of the tumor have YB-1 in the nucleus.

Embodiment 52: The method of any one of Embodiments 1 to 51, wherein the cells of the tumor have YB-1 in the nucleus after induction.

Embodiment 53: The method of Embodiment 52, wherein the transport of YB-1 into the nucleus is triggered by at least one measure selected from the group comprising irradiation, administration of cytostatics and hyperthermia.

Embodiment 54: The method of Embodiment 53, wherein the measure is applied to a cell, an organ or an organism, preferably an organism in need thereof, more preferably an organism suffering from the tumor.

Embodiment 55: The method of claims 1 to 54, wherein the tumor is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

In a tenth aspect, the present invention also relates to the use of a composition for the manufacture of a medicament, wherein the composition is a composition as disclosed in connection with the first aspect of the present invention, including any embodiment thereof, and the medicament is for the treatment and/or prevention of a disease as specified in connection with the second aspect of the present invention, including any embodiment thereof.

In an eleventh aspect, the present inventions also related to the use of an adenovirus for the manufacture of a medicament, wherein the adenovirus is an adenovirus as disclosed in connection with the third aspect of the present invention, including any embodiment thereof,

and the medicament is for the treatment and/or prevention of a disease as specified in connection with the third aspect of the present invention, including any embodiment thereof.

In a twelfth aspect, the present inventions also related to the use of a CDK4/6 inhibitor for the manufacture of a medicament, wherein the CDK4/6 inhibitor is a CDK4/6 inhibitor as disclosed in connection with the fourth aspect of the present invention, including any embodiment thereof, and the medicament is for the treatment and/or prevention of a disease as specified in connection with the fourth aspect of the present invention, including any embodiment thereof.

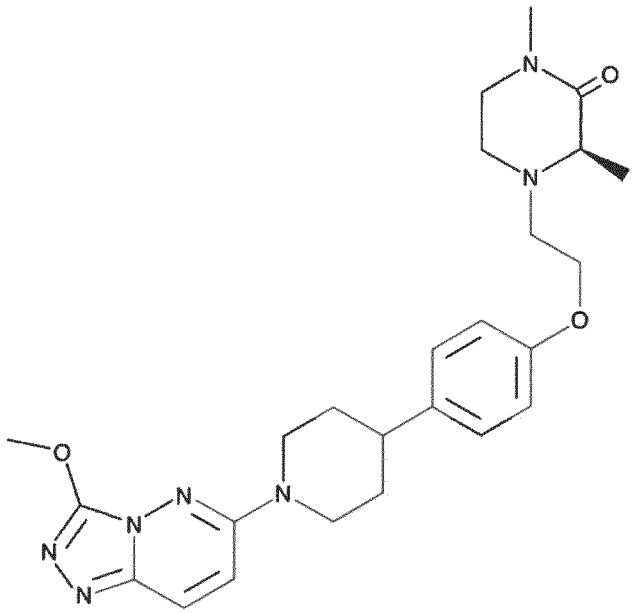
In an 13th aspect, the present inventions also related to the use of a PARP inhibitor for the manufacture of a medicament, wherein the PARP inhibitor is a PARP inhibitor as disclosed in connection with the fifth aspect of the present invention, including any embodiment thereof, and the medicament is for the treatment and/or prevention of a disease as specified in connection with the fifth aspect of the present invention, including any embodiment thereof.

In a 14th aspect, the present inventions also related to the use of a bromodomain inhibitor for the manufacture of a medicament, wherein the bromodomain inhibitor is a bromodomain inhibitor as disclosed in connection with the sixth aspect of the present invention, including any embodiment thereof, and the medicament is for the treatment and/or prevention of a disease as specified in connection with the sixth aspect of the present invention, including any embodiment thereof.

In a 15th aspect, the present inventions also related to the use of nutlin or a derivative thereof for the manufacture of a medicament, wherein the nutlin or nutlin derivative is nutlin or a nutlin derivative as disclosed in connection with the seventh aspect of the present invention, including any embodiment thereof, and the medicament is for the treatment and/or prevention of a disease as specified in connection with the seventh aspect of the present invention, including any embodiment thereof.

In an embodiment of each and any aspect as disclosed herein, including any embodiment thereof, a CDK4/6 inhibitor is an agent which inhibits or is capable of inhibiting CDK4/6 thus causing an arrest of an accordingly treated cell in the G1 phase.

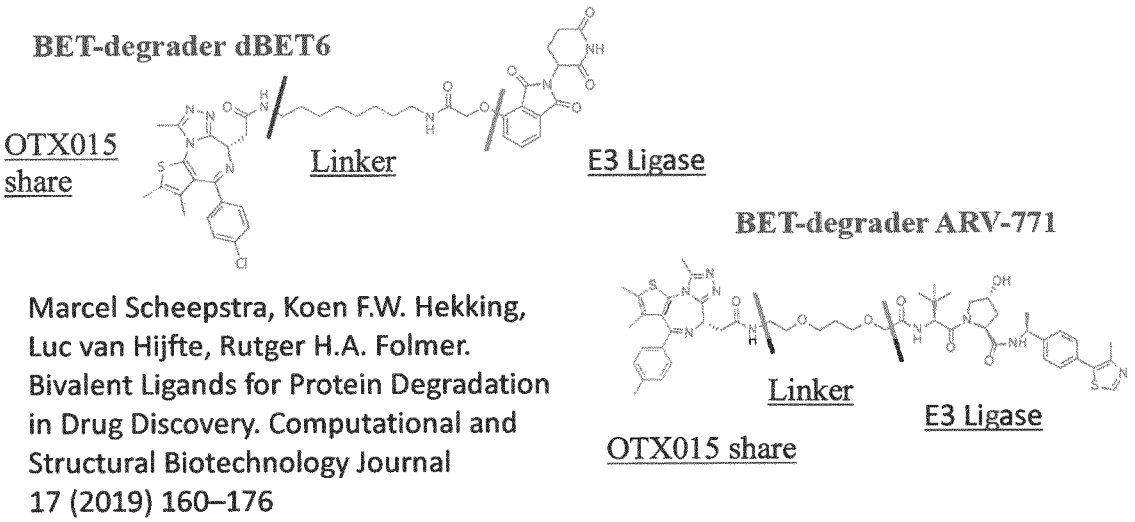
In an embodiment of each and any aspect of the present invention, including any embodiment thereof, the bromodomain inhibitor is a bivalent bromodomain inhibitor, more preferably the bromodomain inhibitor is AZD5153 of the following formula



:

In an embodiment of each and any aspect of the present invention, including any embodiment thereof, the bromodomain inhibitor is a BET degrader, preferably the bromodomain inhibitor is selected from the group comprising dBET6 of the following formula and ARV771 of the following formula:

General structure of BET-degraders



Marcel Scheepstra, Koen F.W. Hekking, Luc van Hijfte, Rutger H.A. Folmer. Bivalent Ligands for Protein Degradation in Drug Discovery. Computational and Structural Biotechnology Journal 17 (2019) 160–176

(see, e.g. Scheepstra M et al. (Computational and Structural Biotechnology Journal 17(2019) 160-179).

It will be acknowledged by a person skilled in the art that each and any embodiment of one aspect of the present invention is also an embodiment of each and any of the other aspects of the present invention, including any embodiment thereof.

Without wishing to be bound by any theory, the present inventors have surprisingly found that combining an oncolytic virus, preferably an oncolytic adenovirus, with a CDK4/6 inhibitor increases the efficacy of tumor therapy based on such oncolytic adenovirus. More specifically, the CDK4/6 inhibitor is assumed to inhibit E2F1 (also referred to as E2F-1 herein) thus reducing its effective concentration, preferably in tumor cells, and synchronizes G1 arrest in cells. Because of this, more infected cells can complete the entire viral life cycle.

Based on the evidence and insights provided herein, a person skilled in the art will understand that any - mutant - adenovirus is suitable for use in the practicing of the instant invention which allows that at least as little as 10 %, 20 % or 30 % of wild type expression and, respectively, activity of E1B55K and E4orf6 is achieved by such adenovirus. It will be appreciated by a person skilled in the art that such mutant adenovirus can be generated by modifying E1A. Exemplary mutant adenoviruses are adenovirus XVir-N-31, dl520, AdΔ24,

AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

Eponymous for adenoviruses is the first isolation of the virus in human tonsils and adenoid tissue in 1953 by Wallace P. Rowe and Robert J. Huebner (Rowe et al., 1953). The family of Adenoviridae comprises five genera, namely Mastadenoviruses, Aviadenoviruses, Siadenoviruses, Atadenoviruses and Ichtadenoviruses (Modrow, 2013). Due to their oncogenicity in newborn rodents, they can be classified into seven subgroups HAdV-A to HAdV-G (Boulanger and Blair, 1991) with altogether 62 serotypes. Thereby, research on oncolytic virotherapy is mainly focusing on Mastadenovirus Type C serotype 5.

The uncoated icosahedral capsid with a size of 80 to 110 nm is comprised of 252 capsomers, that consist of 12 pentons, assembled of a penton basis and spike-like protein structures, called fibers, on the vertices of the capsid and 249 faces, called hexons (Modrow, 2013). The whole lifecycle of adenoviruses, can be subdivided into an early phase with cell entry, nuclear translocation of the viral genome, transcription and translation of early genes and the late phase with transcription and translation of late genes. Late proteins are thereby mainly responsible for assembly of structural proteins and maturation of virions (Russell, 2000). In permissive cells, the early phase takes about 6-8 hours with a following late phase of about 4-6 hours. Attachment occurs via interaction of a knob structure, that is present on every end of the fiber structures with a receptor on the target cells at least for the adenoviruses HAdV-A, -C, -E and -F. Since this receptor was detected as the same one, that is responsible for coxsackie B virus adsorption, the receptor is called coxsackievirus and adenovirus receptor (CAR) (Bergelson, 1997). Additionally, binding on the surface of the target cell is supported by "bridge molecules", soluble proteins in bodily fluids like blood coagulation factors VII and X, that mediates the binding of the fiber proteins of certain adenovirus types (Modrow, 2013). After this adsorption step, an RGD-motif (arginine-glycine-aspartic acid) in the penton base interacts with heterodimeric integrins αvβ3 or αvβ5, that function as co-receptors in this process. This interaction results in internalization of the virus (Wickham et al., 1993). Subsequently, endocytosis via clathrin-mediated internalization in the cytoplasmic membrane occurs and the virus is present in endosomes. After acidification of the endocytic vesicles, the viral fiber protein changes its conformation with resulting destruction of the endosomal

membrane (Greber et al., 1996). Viral particles are now free in the cytoplasm. Via binding of residual particles on dyneins of microtubules, the viral genome is transferred into the nucleus (Modrow, 2013).

The genome of adenoviruses consists of a double-stranded, linear DNA of 36-38 kb length. By interaction of two terminal protein (TP) molecules, that are covalently linked to both 5` ends, a quasi-circular state is formed (Modrow, 2013). In general, five coding regions of the adenoviral genome can be subdivided into the early genes E1-E4, active mainly in the early phase of infection and the late genes (L1-L5), that encode proteins mainly necessary for viral particle formation (Modrow, 2013).

Adenoviral replication is especially dependent on the expression of the early viral gene E2, which is strongly induced by the large E1A protein (E1A13S). The first viral gene post infection to be transcribed is early region 1A (E1A). The primary E1A transcript is processed by differential splicing to yield five distinct messages with sedimentation coefficients of 13S, 12S 11S, 10S, and 9S. The 13S and 12S mRNAs are the most abundant at early times during infection, while the 9S mRNA is the most abundant at late times. The 11S and 10S mRNA are minor species that become more abundant at late times after infection. The 13S, 12S, 11S, 10S and 9S E1A mRNA code for 289 residue (R), 243R, 217R, 171R and 55R proteins respectively, all of which are detectable in vivo with the exception of the 9S product which has only been detected in vitro. In general, adenoviral gene expression is highly regulated in course of infection with a high degree of complexity. Thereby, transcription of the E2 genes which products encode for the viral DNA polymerase and other proteins necessary for efficient viral replication is under control of two promoters, the E2-early and E2-late promoter.

Due to its two overlapping transcriptional control regions, the E2-early promoter can be subdivided into the major promoter starting at position +1 and the minor promoter starting at position -26, both containing a TATA motif (Swaminathan and Thimmapaya, 1996). These motifs serve as binding sites for TATA box-binding proteins (TBP). Moreover, one binding site for the activating transcription factor (ATF) between positions -68 and -77 and two E2F1/DP-1 binding sites (TTTCGCGC), aligned in inverted orientation with respect to each other, are located at position -35 and -63 of the major E2-early promoter (Swaminathan and

Thimmapaya, 1996). The activation of the E2-early promoter through E1A is mainly dependent on the two E2F1-binding sites localized in the major promoter part.

At intermediate stages of infection, after about 6 hpi (hours past infection), expression of E2 genes is controlled by the E2-late promoter. At position nt -33 to -22 of its 157-bp sequence, there is a TATA box, that can be bound and activated by cellular TBP (Swaminathan and Thimmapaya, 1996). Moreover, two SP1 recognition sites and three CCAAT boxes are characteristic for the E2-late promoter.

Since it was shown, that the cellular factor YB-1 is able to bind to inverted CCAAT boxes, interaction between the Y-box binding protein 1 (YB-1) and the E2-late promoter was investigated. Holm et al. showed in 2002, that there is in fact a specific interaction of YB-1 with the Y-boxes (inverted CCAAT-boxes), present in the E2-late promoter with ability to control the activity of this promoter (Holm et al., 2002). To exert its transactivating activity, YB-1 has to be translocated into the nucleus via the adenoviral complex E1B-55k/E4-orf6. These early viral genes are expressed after transactivation of E1A-13S (Frisch and Mymryk, 2002).

The cellular factor YB-1, encoded by the YBX1 gene, is a cold shock domain bearing DNA-binding protein with multiple functions in transcription, splicing, translational control and repair of DNA damages (Kohno et al., 2003). Moreover, it plays an important role in drug-resistance, due to its activation of MDR1 and MRP1 genes that are involved in the development of a multidrug-resistant phenotype in cancer cells (Mantwill et al., 2006). YB-1 expression is induced with subsequent nuclear transport through exposure of extrinsic stress factors like adenoviral infection, chemotherapy or UV radiation (Mantwill et al., 2006).

Transcriptional activation of adenovirus early genes and late genes is pivotal to the viral life cycle. Briefly, the viral life cycle is initiated by the activation of E1A transcription, followed by a cascade of activation of E2, E3 and E4 genes. Finally, the major late promoter (MLP) is activated to coordinate the expression of capsid and accessory proteins involved largely in genome encapsidation (Turner et al 2015). To overcome the block to viral DNA replication present in non-proliferating cells, the virus expresses the early 1A proteins (E1A). These immediate early proteins drive cells into S-phase and induce expression of all other viral early

genes. During infection, several E1A isoforms are expressed with proteins of 289, 243, 217, 171, and 55 residues being present for human adenovirus type 5. In the context of infection, the primary driver of viral gene expression is the large E1A 289R protein (Radko et al 2015).

Upon infection, expression of the adenoviral E1A protein promotes cell cycle progression from G0/G1 phase into S-Phase and viral replication even in terminally differentiated epithelial cells, the major target of human adenoviruses. This process is considered to be essential for adenoviral life cycle.

Adenoviruses have been designed to infect, replicate and kill cancer cells while sparing normal cells. Following infection and replication in tumor cells, oncolytic viruses kill the cells, releasing virions for subsequent cycles of amplification. To achieve replication only into tumor cells, two kinds of genetic modifications have been made, leading to three subclasses of oncolytic adenovirus (also referred to as CRAd herein) have been designed all of which may be used in the practicing of the present invention. Furthermore, oncolytic adenoviruses suitable for use in the practicing of the present invention are, among others, described in WO 2003/099859.

Type I CRAd are characterized by mutations or deletions in the E1 region of the genome, interfering with the inactivation of cell cycle regulators such as p53 and retinoblastoma protein (Rb). As a consequence, type I CRAds replicate in actively dividing tumor cells. For example, Onyx-015, also known as dl1520, which is unable to express the E1B-55 kDa protein, is unable to inactivate p53 and avoid p53-induced cell cycle arrest. Several studies attributed the molecular basis of Onyx-015 selectivity to the lack of expression of p53 or one of genes involved in p53 pathway. However, O'Shea et al. showed that late viral RNA export, rather than p53 inactivation, determines Onyx-015 virus selectivity. Other type I CRAds with deletions in the E1A region are unable to bind Rb and trigger S phase entry. For example, dl922-947 and Δ24 contain a 24 nucleotide deletion in CR2 domain of E1A region, abrogating E1A–Rb interaction. As a result, these viruses replicate mainly in tumor cells where free, unbound E2F1 is available.

Another way to restrict adenoviral replication to tumor cells is to regulate the transcription of viral genes required for viral replication. In type II CRAds, the genome is placed under the control of a tumor-specific promoter. Those promoters were derived from genes known to be preferentially expressed in some tumors compared to normal tissue (e.g., telomerase or cyclo-oxygenase II); or that are overexpressed in tumors (e.g., prostate specific antigen, PSA or α-foetoprotein, AFP) compared to normal tissues. In type III CRAds such as XVir-N-31 (Ad-Delo3-RGD) is characterized by deletion of the transactivation domaim CR3 in the E1A13S protein. XVir-N-31 is replication defective adenoviruses in normal cells. XVir-N-31 restores its replication competence by the presence of the cellular multifunctional protein YB-1 in the nucleus. Accordingly, CRAds are only capable to replicate in tumor cells and thus ultimately lysing them. Neither mutations of p53, nor ras nor RB are effective to complement the replication deficiency of XVir-N-31. XVir-N-31 lack E1A13S, consequently the E1B55k protein and the E4orf6 protein are not expressed. This deficiency is complemented by the presence of YB-1 in the nucleus of tumor cells which triggers the expression of E1B55k and E4orf6 independently of E1A13S. Once induced by the presence of YB-1 in the nucleus, E1B55k and E4orf6 transfer further cellular YB-1 into the nucleus propelling viral replication.

The cell cycle progresses sequentially through the gap 1 (G1), synthesis (S), gap 2 (G2) and mitosis (M) stages. This progression is regulated via a complex signaling network. The CDK (cyclin-dependent kinase) proteins, CDK1, CDK2, CDK4 and CDK6, are major regulators of cell cycle progression when complexed with specific cyclin proteins. Constitutive expression of CDKs and temporal control of various cyclins enables the regulation of specific cell cycle phases by distinct cyclin-CDK complexes. CDK activity is negatively regulated by several inhibitory proteins. The various aspects of CDK biology and function have been previously reviewed comprehensively.

CDK4 and CDK6, which show structural and functional homology, regulate the transition of quiescent cells in the G1 phase into the S phase when complexed with cyclin D proteins. Cyclin D proteins have three subtypes, cyclin D1-3, and accumulate in the presence of mitogenic stimuli. Negative regulators of CDK4/6 include the inhibitor of CDK4 (INK4) proteins, p16INK4A, p15INK4B, p18INK4C and p19INK4D, which inhibit CDK4/6 activity

either by reducing their binding with cyclin D1 or by directly occupying their catalytic domains.

The kinase activity of CDK4/6 leads to the phosphorylation of members of the retinoblastoma (Rb) protein family including Rb, p107 and p130, which results in their functional inactivation. In quiescent cells, active hypophosphorylated Rb binds to members of the E2F1 transcription factor family that form a complex with DP-1/2, together with other co-repressors and suppresses E2F1 function (Rubin et al 2005). Upon phosphorylation, Rb dissociates from this complex and allows the transcription of E2F1 target genes including cyclin A, cyclin E and DHFR, among others, which are required for the transition of the cell cycle into the S phase. Hence, inhibition of CDK4/6 activity leads to Rb dephosphorylation and repression of E2F1 activity, which promotes a G0/G1 arrest. This has fueled the development of CDK4/6 inhibitors as target therapy in cancer cells.

The disruption of the CDK4/6-Rb signalling pathway and an uncontrolled G1-S transition of the cell cycle is a common feature of cancer cells. This can occur due to various molecular alterations including loss of function mutations or deletions of the RB1 gene (encoding for Rb), CDKN2A (encoding for p16INK4A and p14ARF) or CDKN2B (encoding for p15INK4B). Such deregulation can also result from amplification or activating mutations in CCND1 (encoding for cyclin D1), E2F1-3, CDK4, CDK6 or components of various mitogenic signaling pathways such as the PI3K or MAPK pathways.

Several ATP-competitive small molecule CDK inhibitors have been developed. However, first generation inhibitors such as flavopiridol are non-selective and can inhibit multiple CDKs which might result in limited efficacy and high toxicity. Next generation CDK4/6 inhibitors display high selectivity and include palbociclib (PD-0332991 from Pfizer), abemaciclib (LY-2835219 from Eli Lilly) and ribociclib (LEE011 from Novartis) and Trilaciclib (G1T28). These CDK4/6 inhibitors have been tested pre-clinically in in vitro and in vivo models of several cancer entities including leukemia, breast cancer, melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer and metastatic breast cancer (mBC). In most studies they have demonstrated a consistent molecular and functional phenotype with a dose-dependent reduction in Rb phosphorylation, protein expression and transcription of E2F1 target genes,

which correlates with a G0/G1 arrest and inhibition of cell proliferation. Additionally, all these reports demonstrate that Rb expression is a pre-requisite for sensitivity to these inhibitors.

CDK4/6 inhibitors such as PD-0332991, result in a dose dependent reduction in total Rb protein that correlated with a decrease in phosphorylated Rb. This decrease in total Rb correlates partially with a reduction in RB1 transcript levels and transcription of E2F1 target genes CCNA2 and CCNE2. Also, E2F1 expression level is significantly downregulated.

CDK4/6 inhibitors suitable for use in the practicing of the present invention are disclosed in Fig. 25.

As evident from the example part, any CDK4/6 inhibitor is suitable for use in combination with a virus, preferably an adenovirus and more preferably an oncolytic adenovirus, whereby the CDK4/6 inhibitor causes G1 arrest of cells and inhibits E2F1, more specifically, E2F1 activity.

It will be appreciated by a person skilled in the art that any CDK4/6 inhibitor is used in a therapeutically effective concentration.

PARP1 is a protein that is important for repairing single-strand breaks ('nicks' in the DNA). In mammals, 17 PARP family members have been discovered, and only 6 of these synthesize poly ADP-ribose (pADPr). PARP1, PARP2, and PARP3 have roles in DNA repair. PARP1 binds to DNA that has suffered from single-stranded breaks (SSBs) and double-stranded breaks (DSBs). PARP1 then undergoes a conformational change that aligns key amino acid residues in the active site, thereby increasing its activity. Once PARP1 is activated, it synthesizes pADPr, which binds to proteins and alters their function. pADPr is rapidly degraded by pADPr glycohydrolase to ensure that the levels of the pADPr present are reflective of DNA damage and that the response to pADPr is terminated following DNA repair.

By inhibiting DNA repair pathways, PARP1 inhibitors cause an increase in single-stranded breaks within DNA. This DNA damage is unrepaired and carried into daughter cells following replication, as BER is no longer occurring. This leads to an increase in DSBs in tumors that have BRCA1 and BRCA2 mutations (Scott et al. 2015, J Clin Oncol., 33(12): 1397–140). The chemical structures of PARP inhibitors including the PARP drug candidates rucaparib, veliparib and olaparib are shown in Fig. 26 and described in Antolin and Mestres 2014, Oncotarget, 30;5(10):3023-8, including the benzamide moiety that characterizes all PARP inhibitor structures.

In addition, it is well established that YB-1 potentiates PARP activity and decreases the efficacy of PARP1 inhibitors (Alemasova et al.2018, Oncotarget, 34, 23349-65), suggesting that YB-1 dependent oncolytic adenovirus in combination with both CDK 4/6 Inhibitors and PARP-Inhibitors will work synergistically in cancer cell killing. Olaparib and BMN673 (Talazolarib developed from Pfizer, USA, Clin Cancer Res. 2013, 15;19(18):5003-15) are example of PARP-Inhibitors.

It will be appreciated by a person skilled in the art that any PARP inhibitor is used in a therapeutically effective concentration.

CDK4/6 inhibitors suitable for use in the practicing of the present invention are disclosed in Fig. 25.

Aberrations in the epigenetic landscape are a hallmark of cancer and acetylation of lysine residues is a post-translational modification with broad relevance to cellular signaling and disease biology. Enzymes that 'write' (histone acetyltransferases, HATs) and 'erase' (histone deacetylases, HDACs) acetylation sites are an area of extensive research in current drug development. Recruitment of proteins to macromolecular complexes by acetylated lysine residues is mediated by bromodomains (BRDs), which are evolutionarily highly conserved protein-interaction modules that recognise ε-N-lysine acetylation motifs. The conserved BRD fold contains a deep, largely hydrophobic acetyl lysine binding site, which represents an attractive pocket for the development of small, pharmaceutically active molecules. Proteins that contain BRDs have been implicated in the development of a large variety of diseases.

Recently, two highly potent and selective inhibitors that target BRDs of the BET (bromodomains and extra-terminal) family provided compelling data supporting targeting of these BRDs in cancer. The BET (bromodomain and extraterminal domain) subfamily of bromodomain proteins, composed of BRD2, BRD3, BRD4, and BRDT, perform diverse roles in regulating transcription by RNA polymerase II (POLII) and are an exciting new class of epigenetic drug targets. These proteins facilitate the initiation and elongation phases of transcription by binding to activated chromatin at acetylated lysine residues. The recognition of activated chromatin by these so-called epigenetic "readers" promotes the recruitment of the RNA polymerase II complex to sites of active transcription. The BRD4/P–TEFb interaction is important for rapid transcriptional re-initiation after mitosis (Muller et al., 2011, Expert Rev. Mol. Medicine, 13, e19). P-TEFb was identified and purified as a factor needed for the generation of long run-off transcripts using an in vitro transcription system derived from Drosophila cells. It is a cyclin dependent kinase containing the catalytic subunit, Cdk9, and a regulatory subunit, cyclin T in Drosophila. In humans there are multiple forms of P-TEFb which contain Cdk9 and one of several cyclin subunits, cyclin T1, T2, and K. P-TEFb associates with other factors including the bromodomain protein BRD4, and is found associated with a large complex of proteins called the super elongation complex (Yang Z, et al.,2005. Mol Cell; 19:535-45; Fu et al., 1999, J Biol Chem., 274:34527-30).

JQ-1 (thieno-triazolo-1-4-diazepine) is a potent inhibitor of the BET family of bromodomain proteins which include BRD2, BRD3, BRD4 (Filippakopoulos et al., 2010 Nature 468, 1067-1073). JQ-1 prevent interaction between the bromodomain and the acetyl group, causing the downregulation of certain genes. Further BET bromodomain Inhibitors including OTOX15, BAY1238097, GSK2820151, I-BET762 and PLX51107 have been described (Perez-Salvia and Esteller 2017, EPIGENETICS, 12, 323-339; Brandt et al., 2015ACS Chem. Biol., 10, 22−39). JQ-1 is structurally related to benzodiazepines. The formula is C23H25ClN4O2S.

Recently, it has been shown that the BET inhibitor JQ-1 facilitates adenovirus infection and adenoviral vector-mediated gene delivery. Treatment of cells with JQ-1 induces an increase in BRD4 association with CDK9, a subunit of P-TEFb of transcription elongation. However, as stated in the paper, further studies are required to delicate the mechanism by which BED4 utilizes to regulate adenovirus infection and transgene expression (Baojie Lv et al 2018,

Scientific reports, 8, 11554). Importantly viral replication and virus transcription were not investigated. However, it is known, that CDK9 stimulates released of paused polymerase and activates transcription by increasing the number of transcribing polymerases and thus the amount of mRNA synthesis per time (Gressel et al. 2017, eLife, 6, e29736). In addition, it was shown, that BET inhibitor resistance can be overcome by CDK 4/6 inhibitors (Jin et al. 2018, Mol Cell;71(4):592-605). Recently it was demonstrated a dramatic increase in P-TEFb-Brd4 interaction from late mitosis to early G1 phases of cell cycle and active recruitment of P-TEFb to the chromosomes, followed by initiation of transcription of key genes for G1 progression. Importantly, depletion of Brd4 abrogated the whole process by reducing transcription of essential G1 genes, leading to G1 cell cycle arrest and apoptosis (Yang et al., 2008, Mol Cell Biol., 28:967-976, Kohoutek, 2009, Cell Division, 4. 19).

However, nothing is known about using YB-1 dependent oncolytic adenoviruses in conjunction with CDK 4/6 inhibitors and BET inhibitors.

It will be appreciated and is within the present invention that other bromodomain inhibitors will be equally suitable for use in triple therapy using a virus, preferably an adenovirus, more preferably an oncolytic adenovirus such as XVir-N-31, and a CDK4/6 inhibitor.

It will be appreciated by a person skilled in the art that any bromodomain (Bet) inhibitor is used in a therapeutically effective concentration.

Bromodomain inhibitors suitable for use in the practicing of the present invention are disclosed in Fig. 27.

The role of MDM2 on p53 and E2F1

The p53 and retinoblastoma (Rb) proteins are two key tumor suppressors. Mutations in one or both are found in all human cancer tumors and both have been extensively studied as potential therapeutic targets in drug development programs. As p53 and E2F1 are pivotal regulators of cell proliferation and viability (cell death), their abundance and activity are tightly regulated. The Mouse Double Minute 2 protein (MDM2) also known as E3 ubiquitin-protein ligase seems to regulate both MDM2-p53 and the Rb–E2F1 pathway. Since Rb and p53 are not able

to interact directly, it is suggested that MDM2 is a bridge between Rb and p53 (Polager and Ginsberg, Nat Rev Cancer 2009, 9, 738-48). Further supporting the role of MDM2 as an oncogene, several human tumor types have been shown to have increased levels of MDM2, including soft tissue sarcomas and osteosarcomas as well as breast tumors. The MDM2 oncoprotein ubiquitinates and antagonizes p53 but may also carry out p53-independent functions. MDM2 directly binds to p53 and inhibits its transcriptional activity. In addition, as a p53-selective E3 ubiquitin ligase, MDM2 promotes p53 ubiquitylation and targets it for proteasomal degradation (Eischem et al, Hum. Mutant. 20014, 35, 728-737). However, there exist evidence that MDM2 also is required for survival of p53-defecient cancer cells (Feeley et al, Cancer Res 2017, 77, 3823-3833).

In line with a positive effect of MDM2 on the E2F1 pathway, MDM2 can physically interact with Rb, E2F1 and the heterodimeric partner of E2F1, DP1, to promote the G1/S cell cycle transition. Accordingly, MDM2 interaction with E2F1 or DP1 can stimulate transcription of E2F1 target genes that are involved in cell cycle progression. Moreover, targeting of E2F1 for degradation by the F-box protein SKP2 is antagonized by the binding of MDM2. Thus, there exists evidence that MDM2 binding increases E2F1 stability (Zhang et al. Oncogene 2005, 24, 7238-7247), while MDM2 inhibition is associated with a decrease of E2F1 protein level. MDM2, like many other oncoproteins, binds selectively to the hypo-phosphorylated Rb. The interaction between MDM2 and Rb inhibits the Rb-E2F1 complexes formation resulting in suppression of Rb function. In addition, the interaction between RB and MDM2 causes MDM2-mediated Rb degradation. The current facts seem to confirm that MDM2 also (besides p53) is a key negative regulator of Rb (Shi and Gu, Genes Cancer. 2012, 3: 240–248; Yap et al., Oncogene 1999, 18, 7681-7689, Wu et al., JBC 2009, 284, 26315-26321).

Nutlin-3

Nutlins are cis-imidazoline analogs, which inhibit the interaction between MDM2 and tumor suppressor p53. Nutlin-3 is the compound most commonly used in anti-cancer studies. Nutlin small molecules occupy p53 binding pocket of MDM2 and effectively disrupt the p53–MDM2 interaction that leads to activation of the p53 pathway in p53 wild-type cells. In response to nutlin-3 treatment, p53 cancer cells undergo either cell cycle arrest (G0/G1 or G2/S) ore apoptosis. In addition, nutlin-3 can induce differentiation and cellular senescence. It

was shown that an array of factors affects the outcome of Nutlin-3 treatment, including the single nucleotide polymorphism of MDM2, MDM4, p73, ATM, and E2F1. Activation of p53 up-regulates p21 and MDM2, both of which are important regulators for Rb. Recently it was shown that Nultin-3 affects both Rb and E2F1 protein levels and Rb phosphorylation negatively, which significantly affect the cellular responses to Nutlin-3 (Du et al., JBC 2009, 284, 26315-26321).

Although previous studies suggested that the tumorigenicity of MDM2 is due to its negative regulation of p53, the p53-independent interactions may be equally as important. During recent studies utilizing MDM2 inhibitors, it was noted that E2F transcription factor 1 (E2F1) was down regulated upon inhibition of MDM2, regardless of the p53 status of the cancer. One publication describes the used of antisense RNA to inhibit MDM2 function. They concluded that MDM2 has a role in prostate cancer growth via p53-dependent and p53-independent mechanisms. Furthermore, they show that Bcl2, Rb, pRb and E2F1 protein levels were decreased, whereas p21 was increased (Zhang et al 2003, PNAS 2003,100, 11636-11641). However, it was shown that MDM2 prolongs the half-life of the E2F1 protein by inhibiting its ubiquitination. MDM2 displaces SCF(SKP2), the E2F1 E3 ligase. Direct binding between MDM2 and E2F1 is necessary for the negative effects of MDM2 on E2F1 ubiquitination, and deletion of the MDM2 nuclear localization signal does not result in loss of the ability to increase the E2F1 protein level. The downregulation of E2F1 upon MDM2 inhibition was not due to either pRB or p14(Arf). In addition, E2F1 was responsible for at least part of the inhibition of cell proliferation induced by MDM2 knockdown. In conclusion, the present study provides evidence that stabilization of the E2F1 protein is likely another p53-independent component of MDM2-mediated tumorigenesis (Zhang et al., Oncogene 2005, 24, 7238-7247).

Clearly the Rb–E2F1 and MDM2–p53 pathways, along with the multifaceted crosstalk between them, are crucial regulators of cell cycle progression and viability. Clinical studies using MDM2-targeting drugs, however, have currently not met the initial expectations, at least not when used as single drugs. This has spurred the search for optimized combinations of MDM2 inhibitors with other cancer drugs.

Several derivatives of nutlin, have been developed and progressed into human studies (figure 1). These compounds are thought to work best on tumors that contain normal or "wild-type" p53. However, recent results suggest that E2F1 transcriptionally activity is a critical determinant MDM2 antagonist-induced apoptosis and p73 is important for E2F1 mediated apoptosis induced by Nutlin-3 (Burgess et al., Frontiers in Oncology 2016, 6, article 7; Skalniak et al., 2019, 29, 151-170; Kitagawa et al., Oncogene 2008, 27, 5303-5314).

Nutlin and derivatives thereof (NVP-HDM201, Idasanutlin, AM-8553, SAR405838, Nutlin-3a, AMG232) are shown in Fig. 43.

The tumours which can in particular be treated by the viruses and thus combinations of the present invention described herein are preferably those tumours which are selected from the group comprising tumours of the nervous system, ocular tumours, tumours of the skin, tumours of the soft tissue, gastrointestinal tumours, tumours of the respiratory system, tumour of the skeleton, tumours of the endocrine system, tumours of the female genital system, tumours of a mammary gland, tumours of the male genital system, tumours of the urinary outflow system, tumours of the haematopoietic system including mixed and embryonic tumours, and leukemia. It is within the present invention that these tumours are in particular resistant tumours as in particular defined herein.

The group of tumors of the nervous system preferably comprises:

1. Tumors of the skull as well as of the brain (intracranial), preferably astrocytoma, oligodendroglioma, meningioma, neuroblastoma, ganglioneuroma, ependymoma, schwannoglioma, neurofibroma, haemangioblastoma, lipoma, craniopharyngioma, teratoma and chordoma;

2. Tumors of the spinal cord and of the vertebral canal, preferably glioblastoma, meningioma, neuroblastoma, neurofibroma, osteosarcoma, chondrosarcoma, haemangiosarcoma, fibrosarcoma and multiple myeloma; and

3. Tumors of the peripheral nerves, preferably schwannoglioma, neurofibroma, neurofibrosarcoma and perineural fibroblastoma.

The group of the ocular tumors preferably comprises:

1. Tumors of the eyelids and of the lid glands, preferably adenoma, adenocarcinoma, papilloma, histiocytoma, mast cell tumor, basal-cell tumor, melanoma, squamous-cell carcinoma, fibroma and fibrosarcoma;

2. Tumors of the conjunctiva and of the nictitating membrane, preferably squamous-cell carcinoma, haemangioma, haemangiosarcoma, adenoma, adenocarcinoma, fibrosarcoma, melanoma and papilloma; and

3. Tumors of the orbita, the optic nerve and of the eyeball, preferably retinoblastoma, osteosarcoma, mast cell tumor, meningioma, reticular cell tumor, glioma, schwannoglioma, chondroma, adenocarcinoma, squamous-cell carcinoma, plasma cell tumor, lymphoma, rhabdomyosarcoma and melanoma.

The group of skin tumors preferably comprises:

Tumors of the histiocytoma, lipoma, fibrosarcoma, fibroma, mast cell tumor, malignant melanoma, papilloma, basal-cell tumor, keratoacanthoma, haemangiopericytoma, tumors of the hair follicles, tumors of the sweat glands, tumors of the sebaceous glands, haemangioma, haemangiosarcoma, lipoma, liposarcoma, malignant fibrous histiocytoma, plasmacytoma and lymphangioma.

The group of tumors of the soft-tissues preferably comprises:

Tumors of the alveolar soft-tissue sarcoma, epithelioid cell sarcoma, chondrosarcoma of the soft-tissue, osteosarcoma of the soft-tissues, Ewing's sarcoma of the soft-tissues, primitive neuroectodermal tumors (PNET), fibrosarcoma, fibroma, leiomyosarcoma, leiomyoma, liposarcoma, malignant fibrous histiocytoma, malignant haemangiopericytoma, haemangioma, haemangiosarcoma, malignant mesenchymoma, malignant peripheral nerve

sheath tumor (MPNST, malignant schwannoglioma, malignant melanocytic schwannoglioma, rhabdomyosarcoma, synovial sarcoma, lymphangioma and lymphangiosarcoma.

The group of gastrointestinal tumors preferably comprises:

1. Tumors of the oral cavity and of the tongue, preferably squamous-cell carcinoma, fibrosarcoma, Merkel cell tumor, inductive fibroameloblastoma, fibroma, fibrosarcoma, viral papillomatosis, idiopathic papillomatosis, nasopharyngeal polyps, leiomyosarcoma, myoblastoma and mast cell tumor;

2. Tumors of the salivary glands, preferably adenocarcinoma;

3. Tumors of the oesophagus, preferably squamous-cell carcinoma, leiomyosarcoma, fibrosarcoma, osteosarcoma, Barrett carcinoma and paraoesophageal tumors;

4. Tumors of the exocrine pancreas, preferably adenocarcinoma; and

5. Tumors of the stomach, preferably adenocarcinoma, leiomyoma, leiomyosarcoma and fibrosarcoma.

The group of the tumors of the respiratory system preferably comprises:

1. Tumors of the nose and nasal cavity, of the larynx and of the trachea, preferably squamous-cell carcinoma, fibrosarcoma, fibroma, lymphosarcoma, lymphoma, haemangioma, haemangiosarcoma, melanoma, mast cell tumor, osteosarcoma, chondrosarcoma, oncocytoma (rhabdomyoma), adenocarcinoma and myoblastoma; and

2. Tumors of the lung, preferably squamous-cell carcinoma, fibrosarcoma, fibroma, lymphosarcoma, lymphoma, haemangioma, haemangiosarcoma, melanoma, mast cell tumor, osteosarcoma, chondrosarcoma, oncocytoma (rhabdomyoma), adenocarcinoma,

myoblastoma, small-cell carcinoma, non-small cell carcinoma, bronchial adenocarcinoma, bronchoalveolar adenocarcinoma and alveolar adenocarcinoma.

The group of the skeleton tumors preferably comprises:

osteosarcoma, chondrosarcoma, parosteal osteosarcoma, haemangiosarcoma, synovial cell sarcoma, haemangiosarcoma, fibrosarcoma, malignant mesenchymoma, giant-cell tumor, osteoma and multilobular osteoma.

The group of the tumors of the endocrine system preferably comprises:

1. Tumors of the thyroid gland/parathyroid, preferably adenoma and adenocarcinoma;

2. Tumors of the suprarenal gland, preferably adenoma, adenocarcinoma and pheochromocytoma (medullosuprarenoma);

3. Tumors of the hypothalamus/hypophysis, preferably adenoma and adenocarcinoma;

4. Tumors of the endocrine pancreas, preferably insulinoma (beta cell tumor, APUDom) and Zollinger-Ellison syndrome (gastrin secernent tumor of the delta cells of the pancreas); and

5. as well as multiple endocrine neoplasias (MEN) and chemodectoma.

The group of the tumors of the female sexual system tumors preferably comprises:

1. Tumors of the ovaries, preferably adenoma, adenocarcinoma, cystadenoma, and undifferentiated carcinoma;

2. Tumors of the uterine, preferably leiomyoma, leiomyosarcoma, adenoma, adenocarcinoma, fibroma, fibrosarcoma and lipoma;

3. Tumors of the cervix, preferably adenocarcinoma, adenoma, leiomyosarcoma and leiomyoma;

4. Tumors of the vagina and vulva, preferably leiomyoma, leiomyosarcoma, fibroleiomyoma, fibroma, fibrosarcoma, polyps and squamous-cell carcinoma.

The group of tumors of the mammary glands preferably comprises:

fibroadenoma, adenoma, adenocarcinoma, mesenchymal tumora, carcinoma, carcinosarcoma.

The group of the tumors of the male sexual system preferably comprises:

1. Tumors of the testicles, preferably seminoma, interstitial-cell tumor and Sertoli cell tumor;

2. Tumors of the prostate, preferably adenocarcinoma, undifferentiated carcinoma, squamous-cell carcinoma, leiomyosarcoma and transitional cell carcinoma; and

3. Tumors of the penis and the external gentials, preferably mast cell tumor and squamous-cell carcinoma.

The group of tumors of the urinary outflow system preferably comprises:

1. Tumors of the kidney, preferably adenocarcinoma, transitional cell carcinoma (epithelial tumors), fibrosarcoma, chondrosarcoma (mesenchymal tumors), Wilm`s tumor, nephroblastoma and embryonal nephroma (embryonal pluripotent blastoma);

2. Tumors of the ureter, preferably leiomyoma, leiomyosarcoma, fibropapilloma, transitional cell carcinoma;

3. Tumors of the urinary bladder, preferably transitional cell carcinoma, squamous-cell carcinoma, adenocarcinoma, botryoid (embryonal rhabdomyosarcoma), fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, papilloma and haemangiosarcoma; and

4. Tumors of the urethra, preferably transitional cell carcinoma, squamous-cell carcinoma and leiomyosarcoma.

The group of tumors of the haematopoietic system preferably comprises:

1. Lymphoma, lymphatic leukemia, non-lymphactic leukemia, myeloproliferative leukemia, Hodgkin´s lymphoma, Non-Hodgkin`s lymphoma.

The group of the mixed and embryonal tumors preferably comprises:

Haemangiosarcoma, thymoma and mesothelioma.

Preferably, these tumors are selected from the group comprising breast cancer, ovary carcinoma, prostate carcinoma, osteosarcoma, glioblastoma, melanoma, small-cell lung carcinoma and colorectal carcinoma. Further tumors are those which are resistant as described herein, preferably those which are multiple resistant, particularly also those tumors of the group described above.

It is also within the present invention that subjects to which the combination of the invention is to be administered are identified and screened, respectively. Such identification of patients who may benefit from the present invention in its diverse aspects, is based on the detection of YB-1 in the nucleus of a sample of a subject.

In an embodiment, the examination of the tumor tissue is done by using an agent which is selected from the group comprising antibodies against YB-1, aptamers against YB-1 and spiegelmers against YB-1 as well as anticalines against YB-1. Basically, the same means can be produced for the corresponding markers and used accordingly. The manufacture of antibodies, in particular monoclonal antibodies, is known to the ones skilled in the art. A further means for specific detection of YB-1 or the markers, are peptides which bind with a high affinity to the target structures, in the present case YB-1 or said markers. In the prior art methods are known such as phage-display in order to generate such peptides. Typically, a peptide library is taken as a starting point, whereby individual peptides have a length of from 8 to 20 amino acids and the size of the library is about 102 to 1018, preferably 108 to 1015 different peptides. A special form of target molecule binding polypeptides are the so-called anticalines which are, for example, described in German patent application DE 197 42 706.

A further means for specific binding of YB-1 or the corresponding markers disclosed herein and thus for the detection of cell cyclus independent localisation of YB-1 in the cellular nucleus, are the so-called aptamers, i.e. D-nucleic acids which are present either as RNA or DNA either as a single strand or a double strand and specifically bind to the target molecule. The generation of aptamers is, for example, described in European patent EP 0 533 838. A special form of aptamers are the so-called aptazymes, which, for example, are described by Piganeau, N. et al. (2000), Angew. Chem. Int. Ed., 39, no. 29, pages 4369 – 4373. These are special embodiments of aptamers insofar as they comprise apart from the aptamer part a ribozyme part and get catalytically active upon binding or release of the target molecule binding to the aptamer part and cleave a nucleic acid substrate which goes along with the generation of a signal.

A further form of aptamers are the so-called spiegelmers, i. e. target molecule binding nucleic acids which are made of L-nucleic acids. The method for the manufacture of such spiegelmers is, for example, described in WO 98/08856.

The sample of the tumor tissue can be obtained by puncture or through surgery. The assessment whether YB-1 is localised in the nucleus independent from the cell cycle, is frequently done by using microscopic techniques and/or immuno histoanalysis, preferably using the antibody or any of the other aforementioned means. Further means for detecting YB-1 in the nucleus and in particular for detecting that YB-1 is located there independent from the cell cycle, are known to the one skilled in the art. For example, the localisation of YB-1 can be easily detected in stained tissue sections when screening them. The frequency of the presence of YB-1 in the nucleus already indicates that the localisation is independent from the cell cycle. A further option for cell cycle independent detection of YB-1 in the nucleus resides in the staining against YB-1 and detection whether YB-1 is localised in the nucleus and determination of the phase of the cells. This as well as the detection of YB-1 may also be performed by using the afore-mentioned means directed against YB-1. The detection of the means is done by methods known to the one skilled in the art. By said agents specifically binding to YB-1 and not to any other structures within the sample to be analysed, particularly the cells, their localisation and because of their specific binding to YB-1 also the localisation

of YB-1 can be detected and established by a suitable labelling of the means. Methods for the labelling of said means are known to the ones skilled in the art.

In the following, the present invention shall be further illustrated by reference to the figures and samples from which new features, embodiments and advantages may be taken.

Fig. 1 a is a bar diagram showing relative absorbance as an indicator of cell viability for XVir-N-31 (XVir), wild type adenovirus (WT) and control (Ctrl) when used in combination with CDK4/6 inhibitors LY (LY-2835219), PD (PD-032991) or LEE (LEE011).

Fig. 1b is a bar diagram showing viral titre for XVir-N-31 (XVir) and wild type adenovirus (WT) when combined with CDK4/6 inhibitors LY (LY-2835219), PD (PD-032991) or LEE (LEE011).

Fig. 1c is a bar diagram showing relative fiber DNA for XVir-N-31 (XVir) and wild type adenovirus (WT) when combined with CDK4/6 inhibitors LY (LY-2835219), PD (PD-032991) or LEE (LEE011).

Fig. 2 depicts the result of a Western blot analysis.

Figs 3a-d are bar diagrams.

Figs 4a-d are bar diagrams.

Fig. 5 are bar diagrams.

Fig. 6 is a series of microphotographs.

Fig. 7 is a fluorescence microscopic image of T24 cells infected with an E1-deleted Adenovirus expressing GFP with and without Palbociclib treatment.

Fig. 8 is a bar diagram showing viral DNA replication of Adenovirus dl703 after 48 h using compounds Nutlin 3a, Lee, Cl1040 and Roscovertine.

Figs 9A – C show the result of a Western blot analysis of UMUC cells treated with indicated concentrations of Nutlin-3a and LEE011 (Ribociclib) (Fig. 9A), Roscovitine (Fig. 9B) and CI-1040 (Fig. 9C); Rb means retinoblastoma protein; phRB means phosphorylated retinoblastoma protein; E2F1 means transcription factor E2F1; and GAPDH served as loading control.

Fig. 10 is a bar diagram showing cell cycle distribution in UMUC3 cells, measured 48 hours post treatment, whereby the concentrations of the CDK4/6 inhibitors was as follows: Roscovetine: 10 µM, CI-1040: 1 µM, Nutlin-3a: 10 µM, and LEE011: 10 µM.

Fig. 11 is a panel of microscopic images showing adenovirus hexon gene expression with and without Palbociclib treatment.

Fig. 12 is a bar diagram showing the result of a potency assay of T24 cells exposed to XVir-N-31 alone, with 15 nM PARP inhibitor PARPi, 500 nM PD (Palbociclib) or a combination of 15 nM PARPi and 500 nM PD, as percentage cell survival, whereby the cells were either not infected (left column), infected with an MOI of 10 (middle column) or an MOI of 50 (right column).

Fig. 13 is a panel of pictures showing cultures of SRB-stained T24 cells after treatment with XVir-N-31 (20 MOI), XVir-N-31 and 15 nM PARPi, XVir-N-31 and 500 nM PD, and XVir-N-31, 15 nM PARPi and 500 nM PD, 1 dpi, 2 dpi, 3 dpi, 4 dpi, 5 dpi and 6 dpi.

Fig. 14 is a panel of pictures showing cultures of SRB-stained UMUC cells after treatment with XVir-N-31 (10 MOI), XVir-N-31 and 160 nM PARPi, XVir-N-31 and 400 nM PD, and XVir-N-31, 160 nM PARPi and 400 nM PD, 1 dpi, 2 dpi, 3 dpi, 4 dpi, 5 dpi and 6 dpi.

Fig. 15 is a bar diagram showing the result of a potency assay on T24 cells 5 days post infection with XVir-N-31, the CDK 4/6 inhibitor Palbociclib and the bromodomain inhibitor JQ-1; Y-axis: survival of cells in %.

Fig. 16 is a bar diagram showing the result of a potency assay of SK-N-MC cells 5 days after exposure to XVir-N-31 alone, with 200 nM abemaciclib, 500 nM JQ-1 or a combination of 200 nM abemaciclib and 500 nM JQ-1, as percentage cell survival, whereby the cells were either not infected or infected with an MOI of 5, 10 or 20.

Fig. 17 shows the result of a Western blot analysis of SK-N-MC cells treated with indicated concentrations of CDK 4/6 Inhibitor LY-2835219 (Abemaciclib) and the Wee-Inhibitor MK-1775 (Adavosertib) 24 and 48 Hours post treatment; Rb means retinoblastoma protein; phRB means phosphorylated retinoblastoma protein; E2F1 means transcription factor E2F1; and GAPDH served as loading control.

Fig. 18 shows the result of a potency assay on SK-N-MC cells 5 days post infection with XVir-N-31, the CDK 4/6 inhibitor Abemaciclib and Adavosertib (Wee-inhibitor MK-1775) expressed as percentage of living cells.

Fig. 19 shows cell cycle distribution after treatment of SK-N-MC cells with the indicated inhibitors.

Fig. 20 is a bar diagram showing the effect of E2F1 directed siRNA on E2F1 expression in various cell lines; Y axis: E2F1 expression normalized to actin as % of siCTRL transfected cells.

Fig. 21 is a bar diagram showing that E2F1 inhibition causes increased E2-early expression in T24 cells treated with siRNA-E2F1; Y axis: adenoviral gene expression normalized to actin (in % of siCTRL).

Fig. 22 is a scheme showing location of the primers for determining adenovirus E2-early expression.

Fig. 23 is a representation of the nucleotide sequence of the wild type E2 early promoter adenovirus (above) and a mutant E2 early promoter having mutations at the E2F1-binding sites (below).

Fig. 24 is a bar diagram showing RNA expression in AdWT-RGD and AdE2Fm (also containing the RGD motive in the fibre) infected T24 cells obtained by RT-qPCR at 24 hours post infection; AD-WT gene expression was set to 100%.

Fig. 25 shows various CDK4/6 inhibitors suitable for use in the instant invention.

Fig. 26 shows various PARP inhibitors suitable for use in the instant invention.

Fig. 27 shows various Bet inhibitors suitable for use in the instant invention.

Fig. 28 shows the structure of WT-Ad5 and adenovirus dl520 which is an oncolytic adenovirus expressing only the E1A12 protein, through deletion of the CR3-domain of the E1A gene.

Fig. 29 shows the structure of XVir-N-31 which is characterized by deletion of the E1B19K protein, deletion of 2 kb in E3-region, deletion des E1A13S Protein, and introducing a RGD motif the fiber protein.

Fig. 30 shows the structure of Ad-Delta 24 and Ad-Delta 24-RGD which are also described by Kleijn et al. (Kleijn et al., PLoS One. 2014; 9(5): e97495), and characterized by deletion of the CR2-domain of the E1A gene; it replicate only in tumor cells with deregulated retinoblastoma-pathway (Rb). Ad-Delta 24-RGD contains in addition a RGD motive in the fiber knob, as shown in XVir-N-31. Please note the oncolytic adenovirus dl922-947 is similar to delta24, since the deletion in this virus also is located in the E1A-CR2 domain and is affecting RB-binding (retinoblastoma protein).

Fig. 31 shows the structure of VCN-01 which is a replication-competent adenovirus specifically engineered to replicate in tumors with a defective RB pathway, presents an enhanced infectivity through a modified fiber and an improved distribution through the expression of a soluble hyaluronidase (Pascual-Pasto et al. Sci Transl Med. 2019,11 476). The deletion in E1A in VCN-01 is similar to the deletion in delta24 (deletion of the CR2-domain in E1A). Further, the expression of this E1A protein is regulated by introducing E2F1 binding sites in the E1A promoter. In addition, it contains an RGD motif in the fiber knob and

expresses a soluble hyaluronidase (Martínez-Vélez et al. 2016, Clin Cancer Res. 1;22(9):2217-25. The Oncolytic Adenovirus VCN-01 as Therapeutic Approach Against Pediatric Osteosarcoma).

Fig. 32 shows the structure of E1Adl1107 and E1Adl1101, whereby the deletion of these two oncolytic adenoviruses affects binding to p300 (Histone acetyltransferase p300 also known as p300 HAT or E1A-associated protein p300) or pRb (retinoblastoma protein. (Howe et al., MOLECULAR THERAPY 2000, 2, 485-495)

Fig. 33 shows the structure of oncolytic adenovirus CB016 (and the one of wild type adenovirus 5 (WT-Ad5), where deletion in the E1A-CR2 domain is similar as in Ad-Delta 24. In addition, CB016 contains a deletion in the CR1 domain. In addition, it contains either an RGD motif in the fiber or a fiber from serotype 3 (LaRocca et al., Oral Oncol. 2016, 56, 25-31).

Fig. 34 shows the structure of adenovirus ORCA-010 which contains an E1AΔ24 deletion in the E1A CR2-domain, the potency-enhancing T1 mutation in the E3/19K protein, and the infectivity-enhancing fiber RGD modification (Dong et al., Hum Gene Ther. 2014 Oct 1; 25(10): 897–904).

Fig. 35 is a bar diagram showing the results of a potency assay expressed as cell survival in % to determine cell killing effect of XVir-N-31 alone or in combination with Palbociclib alone, with Talazoparib alone or both Palbociclib and Talazoparib ("combination") in UMUC-3 cells 5 days post infection (5 dpi), whereby MOI of XVir-N-31 was 10, 20 or 50.

Fig. 36 is a bar diagram showing the results of a potency assay expressed as cell survival in % to determine cell killing effect of XVir-N-31 alone or in combination with Palbociclib alone, with Talazoparib alone or both Palbociclib and Talazoparib ("combination") in T24 cells 4 days post infection (4 dpi), whereby MOI of XVir-N-31 was 10, 50 or 100.

Fig. 37 is a bar diagram showing the results of a potency assay expressed as cell survival in % to determine cell killing effect of XVir-N-31 alone or in combination with Palbociclib alone, with Talazoparib alone or both Palbociclib and Talazoparib

("combination") in 253J cells 5 days post infection (5 dpi), whereby MOI of XVir-N-31 was 10, 20 or 50.

Fig. 38 is a bar diagram showing the % of cells in cell cycle phases G0/G1, S and G2 of T24 cells (left) and UMUC-3 cells (right), 48 hours post infection with Talazoparib alone, Palbociclib alone or both Talazoparib and Palbociclib ("combination").

Fig. 39 is a bar diagram showing the percentage of surviving cells in a potency assay on A673 cells 4 days post infection with XVir-N-31 at 5, 10 and 20 MOI, the CDK 4/6 inhibitor Abemaciclib at 200 nM and the bromodomain inhibitor JQ-1 at 200 nM.

Fig. 40 is a panel of pictures showing cultures of SRB-stained Cal-33 cells after treatment with XVir-N-31 (1, 5 and 10 MOI), 100 nM Palbociclib alone, 100 nM JQ-1 alone or in combination (both Palbociclib and JQ-1) 4 days post infection.

Fig. 41 is a bar diagram showing the result of the potency test as depicted in Fig. 40 expressed as cell survival in % 4 days after infection of Cal-33 cells at an XVir-N-31 MOI of 5 using 100 nM Palbociclib, 100 nm JQ-1 or a combination of both Palbociclib and JQ-1.

Fig. 42(A,B) are bar diagrams showing XVir-N-31 replication expressed as "relative fibre" in Cal-33 cells 24 hours (Fig. 42A) and 48 hours (Fig. 42B) after infection. MOI was 10, Palbociclib concentration was 100 nM and JQ-1 concentration was equally 100 nM.

Fig. 43 shows nutlin and derivatives thereof, namely NVP-HDM201, Idasanutlin, AM-8553, SAR405838, Nutlin-3a, AMG232.

Fig. 44 is a panel of pictures showing cultures of SRB-stained T24 cells after treatment with XVir-N-31 alone, XVir-N-1 with 30 µM Nutlin-3a, XVir-N-31 with 500 nM Palbociclib and XVir-N-31 with both Nutlin-3a and Palbociclib, whereby the MOI of XVir-N-31 is 0, 1, 5, 10, 20 or 30.

Fig. 45 is a bar diagram showing the result of the potency test as depicted in Fig. 44 expressed as surviving cells relative to control in %.

Fig. 46 is a panel of pictures showing cultures of SRB-stained T24shRb cells after treatment with XVir-N-31 alone, XVir-N-1 with 30 µM Nutlin-3a, XVir-N-31 with 500 nM Palbociclib and XVir-N-31 with both Nutlin-3a and Palbociclib, whereby the MOI of XVir-N-31 is 0, 1, 5, 10, 20 or 30.

Fig. 47 is a bar diagram showing the result of the potency test as depicted in Fig. 46 expressed as surviving cells relative to control in %.

Fig. 48 is a panel of pictures showing cultures of SRB-stained T24 cells after treatment with XVir-N-31 alone, XVir-N-1 with 10 µM Isadanutlin, XVir-N-31 with 500 nM Palbociclib and XVir-N-31 with both Isadanutlinand Palbociclib, whereby the MOI of XVir-N-31 is 0, 5, 10, 20, 40 and 60.

Fig. 49 is a bar diagram showing the result of the potency test as depicted in Fig. 48 expressed as surviving cells relative to control in %.

Fig. 50 is a panel of pictures showing cultures of SRB-stained T24shRb cells after treatment with XVir-N-31 alone, XVir-N-1 with 10 µM Idasanutlin, XVir-N-31 with 500 nM Palbociclib and XVir-N-31 with both Idasanutlin and Palbociclib, whereby the MOI of XVir-N-31 is 0, 5, 10, 20, 40 and 60.

Fig. 51 is a bar diagram showing the result of the potency test as depicted in Fig. 50 expressed as surviving cells relative to control in %.

Fig. 52 is bar diagrams showing XVir-N-31 replication expressed as "relative fibre" in T24shRb cells (left) and T24 cells (right) after 24 hours and 48 hours, respectively. MOI was 20, Palbociclib concentration was 500 nM and Nutlin-3a concentration was 30 µM.

Fig. 53 shows the result of a Western Blot analysis.

Fig. 54 is a bar diagram showing relative amount of E2F1 protein upon exposure of T24shRB cells to Palbociclib, Nutlin-3a or a combination of both.

Figs. 55(A)-(D) are bar diagrams showing the % of cells in cell cycle phases G0/G1, S and G2 of T24 cells (A), T24shRb cells (B), UMUC-3 cells (C) and RT112 cells (D) upon exposure of the cells to Palbociclib, Nutlin- 3a or a combination of both at the indicated concentrations.

Figs. 56(A)-(D) are bar diagrams showing the % of cells in cell cycle phase G1 of T24 cells (A), T24shRb cells (B), UMUC-3 cells (C) and RT112 cells (D) upon exposure of the cells to Palbociclib, Nutlin- 3a or a combination of both at the indicated concentrations.

Fig. 57 is a bar diagram indicating relative survival of U87 cells post XVir-N-31 infection with 100nM Ribociclib (LEE also referred to as LEE011), with 100 nMJQ1 and with both 100nM Ribociclib (LEE) and 100 nMJQ1, with MOI for XVir-N-31 being 5.

Fig. 58 is a bar diagram indicating relative survival of LN229 cells post XVir-N-31 infection with 100nM Ribociclib (LEE also referred to as LEE011), with 200 nMJQ1 and with both 100nM Ribociclib (LEE) and 200 nMJQ1, with MOI for XVir-N-31 being 20.

Fig. 59 is a bar diagram indicating relative survival of T98G cells post XVir-N-31 infection with 1 μM Ribociclib (LEE also referred to as LEE011), with 200 nMJQ1 and with both 100nM Ribociclib (LEE) and 200 nMJQ1, with MOI for XVir-N-31 being 50.

Fig. 60 is a bar diagram indicating relative amount of adenovirus XVir-N-31 fiber DNA in U87 cells when exposed to Ribociclib (LEE) (500 nM), JQ1 (50 nM) or a combination of Ribociclib (500 nM) and JQ1 24 (50 nM) 24 hours post infection (h.p.i.) with XVir-N-31.

Fig. 61 is a bar diagram indicating relative amount of adenovirus XVir-N-31 fiber DNA in LN229 cells when exposed to Ribociclib (LEE) (500 nM), JQ1 (100 nM) or a combination of Ribociclib (500 nM) and JQ1 (100 nM) 48 hours post infection (h.p.i.) with XVir-N-31.

Fig. 62 is a bar diagram indicating relative amount of adenovirus XVir-N-31 fiber DNA in T98G cells when exposed to Ribociclib (LEE) (1 μM), JQ1 (100 nM) or a combination of Ribociclib (1 μM) and JQ1 (100 nM) 48 hours post infection (h.p.i.) with XVir-N-31.

Fig. 63 shows the result of a Western blot analysis of LN229 cells 24 hours post infection with XVir-N-31 (MOI 20) upon exposure to 500 nM LEE, 200 nM JQ-1 or a combination of both 500 nM LEE and 200 nm JQ-1.

Fig. 64 shows the result of a Western blot analysis of LN229 cells 48 hours post infection with XVir-N-31 (MOI 20) upon exposure to 500 nM LEE, 200 nM JQ-1 or a combination of both 500 nM LEE and 200 nm JQ-1.

Fig. 65 shows the result of a Western blot analysis of LN229 cells 72 hours post infection with XVir-N-31 (MOI 20) upon exposure to 500 nM LEE, 200 nM JQ-1 or a combination of both 500 nM LEE and 200 nm JQ-1.

Fig. 66 shows the result of a Western blot analysis of LN229 cells 72 hours post infection with XVir-N-31 (MOI 20) upon exposure to 500 nM LEE, 200 nM JQ-1 or a combination of both 500 nM LEE and 200 nm JQ-1.

Fig. 67 is an illustration of interacting partners of E1A and location of the conserved region CR1-CR4.

Fig. 68 is a bar diagram showing the relative increase of adenovirus DNA in LN229 cells 24 hours post infection (PI) of adenoviruses AdWT, dl1119, Ad delta 24, XVir-N-31 and AdWT/E2Fm (MOI 20) upon JQ-1 treatment (200 nm).

Fig. 69 is a bar diagram showing the relative increase of adenovirus DNA in LN229 cells 48 hours post infection (PI) of adenoviruses AdWT, dl1119, Ad delta 24, XVir-N-31 and AdWT/E2Fm (MOI 20) upon JQ-1 treatment (200 nm).

Fig. 70 is a diagram showing quantified fibre DNA in UMUC-3 cells (expressed as fibre/actin) normalized to fibre at 4 hours) 24 hours post infection with XVir-N-31 after priming or concurrent treatment with with either 100 nM JQ-1 (left) or 500 nM JQ-1 (right).

Fig. 71 is a diagram indicating particle formation of XVir-N-31 (expressed as PFU/ml) in UMUC-3 cells at 39, 49, 62 or 72 hours post infection (hpi) with and without 500 nM JQ-1.

Fig. 72 is a panel of bright-field photomicrographs of XVir-N-31 infected cells (MOI of 10) after hexon titer test 39, 49, 62 or 72 hours post infection (hpi) without (upper row) and 500 nM JQ-1 (lower row).

Fig. 73 is a Western blot analysis of UMUC-3 cells upon treatment with XVir-N-31 with or without 500 nM JQ-1 at 12 hours post infection, 24 hours post infection, 36 hours post infection and 48 hours post infection illustrating quantified viral expression kinetics.

Fig. 74 is a bar diagram showing the percentage of UMUC-3 cells (left) and RT112 cells (right) in G0/G1 phase, S phase and G2 phase under the influence of 100 nM JQ-1, 300 nM JQ-1, 500 nM Palbociclib, a combination of 100 nM JQ-1 and 500 nM Palboccilib, and a combination of 300 nM JQ-1 and 500 nM Palbociclib.

Fig. 75 shows the result of a Western blot analysis of UMUC-3 cells (left) and RT-112 cells (right) upon exposure to JQ-1 and/or Palbociclib at 0, 0.2 μM, and 0.5μM 24 hours post treatment.

Fig. 76 is a bar diagram showing the effect of XVir-N31 (MOI of 5) on the killing of UMUC-3 cells upon treatment with 200 nM JQ-1, 100 nM Palbociclib, and a combination of 200 nM JQ-1 and 100 nM Palbociclib, each five days post infection.

Fig. 77 is a bar diagram showing the effect of XVir-N31 (MOI of 40) on the killing of RT112 cells upon treatment with 200 nM JQ-1, 300 nM Palbociclib, and a combination of 200 nM JQ-1 and 300 nM Palbociclib, each five days post infection.

Fig. 78 is a bar diagram showing the effect of XVir-N31 (MOI of 40) on the killing of T24 cells upon treatment with 100 nM JQ-1, 200 nM Palbociclib, and a combination of 100 nM JQ-1 and 200 nM Palbociclib, each five days post infection.

Fig. 79 is a bar diagram showing replication of XVir-N-31 in UMUC-3 cells 24 hours post infection with XVir-N-31 (MOI of 10) after priming and concurrent treatment with JQ-1 (300 nM), Palbociclib (100 nM) or a combination of both JQ-1 (300 nM) and Palbociclib (100 nM), whereby replication of XVir-N-31 is quantified as relative fiber DNA level.

Fig. 80 is a bar diagram showing replication of XVir-N-31 in T24 cells 24 hours post infection with XVir-N-31 (MOI of 50) after priming and concurrent treatment with JQ-1 (100 nM), Palbociclib (200 nM) or a combination of both JQ-1 (100 nM) and Palbociclib (200 nM), whereby replication of XVir-N-31 is quantified as relative fiber DNA level.

Fig. 81 is a bar diagram showing replication of XVir-N-31 in RT112 cells 24 hours post infection with XVir-N-31 (MOI of 20) after priming and concurrent treatment with JQ-1 (200 nM), Palbociclib (300 nM) or a combination of both JQ-1 (200 nM) and Palbociclib (300 nM), whereby replication of XVir-N-31 is quantified as relative fiber DNA level.

Fig. 82 is a bar diagram illustrating quantitative average production of virus producing UMUC-3 cells infected with XVir-N-31 (MOI of 9) with 200 nM JQ-1, with 500 nM Palbociclib or a combination of both 200 nM JQ-1 and 500 nM Palbociclib, whereby quantification of average production is indicated as stained cells per field of view (f.o.v).

Fig. 83 is a panel of bright-field photomicrographs of XVir-N-31 infected UMUC-3 cells (MOI of 9) after hexon titer test, whereby the cells were infected with XVir-N-31 alone, a combination of XVir-N-31 and 200 nM JQ-1, a combination of XVir-N-31 and 500 nM Palbociclib, or a combination of XVir-N-31, 200 nM of JQ-1 and 500 nM of Palbociclib.

Fig. 84 is a bar diagram indicating relative survival of UMUC-3 cells post infection with XVir-N-31 (at MOIs of 0, 5 and 10) in combination with indicated BET inhibitors OTX (300 nM), AZD (5 nM), dBet6 (50 nM) and ARV (50 nM).

Fig. 85 is a bar diagram indicating relative survival of RT112 cells post infection with XVir-N-31 (at MOIs of 0, 20 and 50) in combination with indicated BET inhibitors OTX (130 nM), AZD (10 nM), dBet6 (150 nM) and ARV (10 nM).

Fig. 86 is a bar diagram indicating viral replication in UMUC-3 cells 24 hours post infection with XVir-N-31 (MOI of 10) in combination with indicated BET inhibitors (OTX: 50 nM; AZD: 50 nM, dBet: 50 nM, and ARV: 50 nM).

Fig. 87 is a bar diagram indicating viral replication in RT112 cells 24 hours post infection with XVir-N-31 (MOI of 50) in combination with indicated BET inhibitors (OTX: 40 nM; AZD: 15 nM, dBet: 25 nM, and ARV: 15 nM).

Fig. 88 is an illustration of the animal studies indicating the dosing and scheduled application of XVir-N-31 and Ribociclib. Ribociclib succinate (LEE011) was administered daily at 200 mg/kg/ body weight by oral gavage for a total of 5 days (day X until X+4). Dissolvent without LEE011 was applied in PBS and XVir-N-31 only animals. XVir-N-31 was injected into the tumor twice at day X+1 and X+3. All control animals, which did not receive XVir-N-31, received i.t. PBS injections, respectively.

Fig. 89 is a diagram showing volume growth curves of various treatment groups (PBS, LEE, XVir only and combo); each data point shows the mean ± tumor size at indicated days after initiation of treatment.

Fig. 90 is a box plot diagram showing tumor volume [mm^3] for the various treatment groups (PBS number of animals = 5), LEE (PBS number of animals = 6), XVir only (PBS number of animals = 7) and combo (PBS number of animals = 7)) at day 12-21 after initiation of treatment.

Fig. 91 is a bar diagram showing viral genomes in tumors (expressed as fiber/1000 actin) of representative animals receiving combination treatment compared to XVir-N-31 only treatment; assessment 2 days after second i.t. injection of XVir-N31.

Example 1: Materials and Methods

Cell Culture

Human bladder cancer cell lines were cultured under subconfluent conditions in RPMI or DMEM medium (Biochrom AG) at 5% or 10% CO2, respectively, supplemented with 10% FBS (Biochrom AG) and 1% NEA (Biochrom AG). Depending on the cell line and experimental conditions, 0.2-1x106, 0.5-1x105, 0.25-0.5x105, and 500-700 cells were seeded in 10 cm, 6-well, 12-well and 96-well formats, respectively.

Cell lines

HeLaP

HeLa P cells (ATCC CCL-2) are epithelial cells from cervical adenocarcinoma named after the patient Henrietta Lacks. This cell line is the most widely distributed and oldest cell line (Rahbari et al., 2009), since it was the first permanent cell line, established in 1951 (Gey et al., 1952). Cultivation occurred in DMEM (10% FBS, 1% PS) under 10% CO2 conditions at 37°C.

HeLaRDB

HeLaRDB is a sub-cell line of the HeLaP-cell line, with resistance to daunoblastin based on overexpression of the glycoprotein P. The resistance was achieved through cultivation with medium containing this anthracycline. This cytostatic agent intercalates in double-stranded DNA sequences and inhibits cellular transcription and replication (Mizuno et al., 1975). As a result of the stress reactions, caused by daunoblastin treatment, the cellular factor YB-1 shows higher nuclear localization in comparison to the parental cell line (Holm et al., 2004). To maintain the resistance against daunoblastin, the cells were cultured in DMEM (10% FBS, 1% PS) containing 0.25 µg/ml daunoblastin under 10% CO2 conditions at 37°C every 14 days.

A549

A549 cells (ATCC CCL-185) were isolated in 1972 from an adenocarcinoma in the human alveolar basal (Giard et al., 1973). Cultivation occured in Dulbecco's MEM (10% FBS and 1% PS) at 37 °C and 10% CO2.

T24

T24 cells (ATCC HTB-4) derived 1970 of a primary human urinary bladder carcinoma (Bubenik, Baresovà et al., 1973). Due to a point mutation in the HRAS gene (Reddy et al., 1982), the MAPK and PI3K pathway is activated. Moreover, an additional mutation in the gene locus of the tumor suppressor gene p53 is present in this cell line (Pinto-Leite et al., 2014). The cells were cultivated with RPMI containing 10% FCS, 1% PS and 1% non-essential amino acids at 37°C under 5% CO2 conditions.

HEK293

HEK293 cells (ATCC CRL-1573) are human embryonic kidney cells isolated in 1973. Due to a stabile transfection of a 4.5 kb-sized part of the genome of adenoviral serotype 5, which includes the whole E1 region (Graham and Smiley, 1977), this cell line is used for production of E1-deficient adenoviruses and for measurement of virus titer.

Table 1: Primer

Name	Forward primer	Reverse primer	Company
Fiber	AAGCTAGCCCTGCAAACATCA	CCCAAGCTACCAGTGGCAGTA (SEQ ID NO: 1)	Eurofins
E2 early	CCGTCATCTCTACAGCCCAT	GGGCTTTGTCAGAGTCTTGC (SEQ ID NO: 2)	Invitrogen
E2 late	CTTCCTAGCGACTTTGTGCC	GTCAGAGTGGTAGGCAAGGT (SEQ ID NO: 3)	Invitrogen
E1A 12S	CGACGAGGATGAAGTCCTGTGTCTG	CTCAGGATAGCAGGCGCCAT (SEQ ID NO: 4)	Metabion
E1A 12S short	GAGGATGAAGTCCTGTGT	CTCAGGATAGCAGGCGCCAT (SEQ ID NO: 5)	Metabion
E1A 13S	TGTTTGTCTACAGTCCTGTGTCTG	CTCAGGATAGCAGGCGCCAT (SEQ ID NO: 6)	Metabion
E1A 13S short	TTGTCTACAGTCCTGTGT	CTCAGGATAGCAGGCGCCAT (SEQ ID NO: 7)	Metabion

E4orf6 Metabion TCCCTCCCAACA CACAGAGT GACAGGAAACCG TGTGGAAT (SEQ ID NO: 8)

Rb
Life Techno. AGCAACCCTCCTAAACCACT TGTTTGAGGTATCCATGCTATCA (SEQ ID NO: 9)

E2F1
Life Technology ACGCTATGAGACCTCACTGAA TCCTGGGTCAACCCCTCAAG (SEQ ID NO: 10)

E2F2
Life Technology CGTCCCTGAGTTCCCAACC GCGAAGTGTCATACCGAGTCTT (SEQ ID NO: 11)

GAPDH
MWG TGGCATGGACTGTGGTCATGAG ACTGGCGTCTTCACCACCATGG (SEQ ID NO: 12)

Actin
Eurofins TAAGTAGGTGCACAGTAGGTCTGA AAAGTGCAAAGAACACGGCTAAG (SEQ ID NO: 13)

L4 33K
Eurofins GAACCAGGGCCGCCCATACTG GGGCTTTGTCAGAGTCTTGC (SEQ ID NO: 14)

L4 22 K
Eurofins CCGTTAGCCCAAGAGCAAC CGGCCGTGATGGTAGAGAAG (SEQ ID NO: 15)

L4HexAss

Eurofins CTGTGGTACTTCCCAGAGAC CAGGTGAGTTATACCCTGCC (SEQ ID NO: 16)

Virus Characteristics

Ad-WT +AdWT-RGD Wildtype Mastadenovirus, Type C, Serotype 5 and ADWT with additional RGD-fiber motif

AdWT-E2F1mut. Mastadenovirus, Type C, Serotype 5, mutations in both E2F1 binding sites of the E2-early promoter with additional RGD-fiber motif and a 2,7 kb-sized deletion in the E3 region (ΔE3)

XVir-N-31 Mastadenovirus, Type C, Serotype 5 with deletions in the E1B-region (1.716-1915, 200 bp), E3-region (28.132-30.813) and 12 base deletion in the E1A-region. Replicates in cancer cells only displaying nuclear YB-1 expression.

XVir-N-31/E2F1M Mastadenovirus, Type C, Serotype 5 with deletions in the E1B-region (1.716-1915, 200 bp), E3-region (28.132-30.813) and 12 base deletion in the E1A-region. Replicates in cancer cells only displaying nuclear YB-1 expression. mutations in both E2F1 binding sites of the E2-early promoter with additional RGD-fiber motif and a 2,7 kb-sized deletion in the E3 region (ΔE3)

Target gene	siRNA construct	Manufacturer
Control	Control (non-sil.) siRNA, 20 µM	Qiagen, the Netherlands
E2F1	E2F1 (SASI_Hs01_00162220), 10 µM	Sigma, Merck, Germany
YB-1	YBX1 siRNA FlexiTube, 10µM	Qiagen, the Netherlands

Methods

siRNA Transfection

Downregulation of certain genes was performed using siRNA transfection. Thereby, 5 µl Lipofectamin RNAiMAX (Thermo Fischer) reagent was added to 150 µl of Opti-MEM in one tube and 36 pmol of siRNA was combined with 150 µl of Opti-MEM in another tube. After combining the contents of both tubes and brief vortexing, the solution was incubated for 5 minutes at room temperature. 250 µl of the siRNA-lipid complex was then added to the 250.000 – 1.000.000 cells, seeded in 6-well plates on the previous day without changing the medium, reaching a final concentration of siRNA of 30 pmol per well. After 48 hours of incubation at 37°C at 10% CO2 conditions, infection or lysation took place.

RNA-Quantification in combination with siRNA

RNA was also quantified in cells were virus was combined with siRNA transfection. Thereby, 125.000 cells were seeded and transfected on the following day with 30 pmol of siRNA-construct of Ctrl-, YB-1-, and E2F1-siRNA. After 48 hours of incubation, infection took place and lysation occurred 24 hours post infection. The lysates were stored at -20°C.

RNA Isolation

Cells were rinsed with PBS and lysed with lysis buffer (mirVana miRNA isolation kit, Life Technologies) and transferred into 1.5 ml reaction tube. 50 µl of homogenate additive (mirVana miRNA isolation kit, Life Technologies) was added to the lysates, resuspended and incubated for 10 minutes on ice. 500 µl of acid-phenol-chloroform was added, vortexed for approximately 30 seconds and incubated for 2 minutes on ice. After centrifugation for 5 minutes at room temperature at 14.000 g, the aqueous and organic phases are separated. The upper aqueous phase was transferred to a new snap cap and combined and inverted with the equal amount of Isopropanol. After incubation for 10 minutes at room temperature the samples were centrifuged at 4°C and 14.000 g for 30 minutes. Subsequently the supernatant was removed and the RNA pellet was washed with 1 ml 75% ethanol. The samples were briefly centrifuged at 7500 g for 5 minutes at 4°C. After removing the supernatant, the air dried pellet was solved in 20 µl nuclease-free water and incubated for 10 minutes at 55°C and 500 rpm in a thermomixer. Subsequently the RNA concentrations were measured via spectrophotometral meaurement. To avoid amplification of ruts of DNA, a DNAse digestion

was performed. Thereby the Deoxyribonuclease I, Amplification Grade-Kit by Invitrogen by life technologies was used. To 1 µg RNA, 1 µl 10x DNAse I Reaction buffer and 1 µl DNAse I are added and filled with DEPC-treated water to an end volume of 10 µl and incubated for exactly 15 minutes at room temperature. By adding 1 µl of 25 mM EDTA solution, the DNase I is inactivated and thereby the process of DNAse digestion is stopped. The samples were incubated for 10 minutes at 65°C and were then used for reverse transcription.

Reverse Transcription

To rewrite RNA to cDNA the High capacity cDNA Reverse Transcription Kit (Thermo Scientific) was used. 2 µg RNA of the DNA digested samples were added to Mastermix containing transcription buffer, 100 mM dNTPs and RNAse inhibitor in PCR soft tubes. Thereby it had to be considered, that the RNA transcribed via the E2-early and E2-late promoter could not be rewritten by random primers, usually used for reverse transcription, because these random primers would bind to both strands of the double-stranded adenoviral genome. Therefor, the rewriting from RNA to cDNA for the samples used for the E2-early and E2-late quantification was performed by using the specific E2-early reverse primer (Table 1). For the housekeeping gene actin, that was used to normalize the results, the random primer was used.

DNA-Replication Analysis

To investigate viral replication within infected cells DNA-replication analysis was performed. 125.000 cells were seeded in 6-well plates and infected with 10-20 MOI. After 2 respectively 8, 12, 24, 36 and 48 hours post infection, lyzation took place. Thereby, the medium was removed and the adherent cells were washed with 1 ml PBS. After adding 200 µl DNA-lysis buffer, the adherent cells were detached from the plate using a cell scraper. The lysate was then transferred into a snap cap. 3 µl of the enzyme proteinase K was added and incubated at the 56°C and 550 rpm at a thermomixer overnight. On the following day, DNA isolation was performed.

DNA Isolation

For purification of DNA, 200 µl phenol-chloroform-isoamylalcohol was added to the lysate. After vortexing and subsequent incubation for 5 minutes on ice, a phase separation was achieved by centrifugation for 3 minutes at 16430 g at 4°C. The upper aqueous phase was

transferred to a new snap cap, containing 200 µl chloroform and 20 µl cresol red in 10 mM TrisCl for a better visualization of the phases. After vortexing and incubation for 5 minutes for 5 minutes on ice, centrifugation for 3 minutes at 16430 g at 4°C took place. Again, the upper aqueous phase was combined with 800 µl ethanol and 50 µl 3M sodium acetate solution. 2 µl Glycogen was added, to achieve a better precipitation. After short inversion of the tube, the solution was centrifuged for 30 minutes at 16430 g at 4°C. Subsequently, the DNA pellet was covered by 400 µl 70% ethanol and incubated for 10 minutes at room temperature. After centrifugation for 7 minutes at 4760 g at room temperature, the DNA pellets were dried for about 5-10 minutes at 37°C. Subsequently, the pellets were dissolved in 100 µl 0,1xTE-buffer and shaken at 40°C at 400 rpm for approximately 3 hours. When the DNA was completely dissolved, the DNA concentration was measured by means of a spectrophotometer using 2 µl of DNA solution for the measurement and 0,1xTE-buffer as a blank solution. The DNA was then stored at 4°C.

qPCR

For further quantification real time quantitative PCR were used. 5 µl of Template DNA respectively cDNA was used in a final concentration of 10 ng/µl. qPCR was performed using 10 µl Mastermix GoTaq qPCR (Promega Corporation) (7,5 µl Mastermix, 1,5 µl primer, 1 µl H2O) and 5 µl DNA template in a 96-well plate pipetted as duplicates. Relative quantification was performed using the comparative CT method with two normalizer genes. The plate was closed via a foil and centrifuged at room temperature for 2 minutes at 220 g. Then the plate was incubated following a certain temperature-time-program in the thermal cycler. Primer used are listed in table 1. Reactions were carried out on a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories).

qPCR cycling conditions

Fiber: 94°C for 2 minutes, 94 °C for 15 seconds, 60 °C for 15 seconds and 72 °C for 15 seconds, for 45 cycles

Other viral genes: 94°C for 1,5 minutes, 94 °C for 15 seconds, 58 °C for 15 seconds and 72 °C for 15 seconds, for 45 cycles

Rb: 94°C for 2 minutes, 94 °C for 15 seconds, 60 °C for 30 seconds and 72 °C for 1 minute, for 44 cycles

E2F1s: 95°C for 2 minutes, 95 °C for 15 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds, for 40 cycles

Protein isolation

Cells were lysed using an 1% SDS buffer, to achieve the disruption of the nuclear membrane. To avoid denaturation of the proteins, the whole process was performed on ice. After suctioning the medium, the cells were washed twice with cold PBS. The adherent cells of one well of a duplicate approach was lysed with 200 µl of 1% SDS buffer and scraped by means of a cell scraper. The lysate was then transferred to the other well of the duplicate approach and again scraped. The lysate of both wells combined, was then transferred in a snap cap tube. Subsequently the lysates were treated with a syringe, to destroy the viscous DNA and centrifuged for 30 minutes at 4°C with 31000 rpm. Because the proteins are present in the supernatant, the supernatant was transferred into a new snap cap tube and used for further steps.

Protein quantification

To quantify the amount of protein, the bicinchoninic acid (BCA) assay by means of the Pierce TM BCA Protein Kit was performed. Thereby 112,5 µl of the BCA solution A+B (50:1) and 12,5 µl of the sample were added into one well of a 96-well plate and incubated for 30 minutes at 37°C. Dependent on the protein concentration, a staining of the solution resulted. By means of a standard series with known protein concentrations, the protein concentrations of the samples were determined by photometric measurement at 562 nm in the microplate reader.

SDS gel electrophoresis

To separate the proteins in subsequent sodium dodecyl sulfate polyacrylamide gel electrophoresis, the calculated amounts of lysate and lysis buffer were mixed with 15 µl loading buffer-DDT-Mixture (6:1). The protein loading substances were then cooked for five minutes at 100°C. 5 µl of the color protein standard and 40 µl of the samples were then loaded onto the gel. For protein separation with detection of viral proteins a 10% gel was used. To study the downregulated genes via siRNA, 12% gels were used. The composition of the resolving and stacking gels are listed in section Buffers and solutions. For approximately 20 minutes the gel was running in TGS-Buffer at 90 V to concentrate all proteins in one band.

Subsequently the gels run for approximately 60 minutes at 150 V in TGS-Buffer, to separate the proteins by size.

Western Blot

To transfer the proteins from the gel onto a membrane it was blotted using the western blot technique. To activate the hydrophobic PVDF-membrane, it was incubated for about 2 minutes in methanol. Subsequently, the membrane together with the sponges, filter papers and the gel were deposit in blotting buffer. By means of electrophoresis for approximately two hours at 100V at 4°C, the proteins were transferred on the membrane in blotting buffer. To avoid unspecific antibody binding, the membrane was blocked rotating for one hour at room temperature in 10 ml 5% milk powder in TBST for analyzing cellular proteins respectively in 5 ml 5% BSA-TBST for the subsequent use of antibodies detecting viral proteins. After washing the membrane five times in TBST for five minutes each, the membrane was incubated with the primary antibody solution at 4°C rotating overnight. For the antibodies GAPDH, E1A, E1B55K, E2A and E4orf6 this step was performed for one hour at room temperature. The antibodies were thereby diluted with different factors in 5% BSA in TBST with 0,02% sodium azide. After additional five washing steps, the membrane was incubated rotating for 30 Minutes at room temperature in a 1:10.000 dilution of the secondary antibody. The secondary antibody (anti-mouse) for the viral antibodies were diluted in 5% BSA-TBST, all others in 5% milk powder in TBST. Those secondary antibodies are conjugated with a horse-radish peroxidase. After five final washing steps, the membrane was incubated five minutes in Enhanced-Chemi-Luminescence (ECL) solution to visualizing the signal of the peroxidase. For the membranes, incubated with the primary antibodies DP-1 and E2F1 the Amersham ECL Prime Western Blotting Detection Reagent by GE-Healthcare was used to achieve brighter signals, for all others, ECL solutions produced in the lab were used. The composition of ECL A and ECL B, that are mixed shortly before usage 1:1 are listed in section Buffers and solutions. Finally, the proteins could be detected by means of developing the signal on a film.

Antibodies:

Checkpoint kinase 1 (sc-377231, Santa Cruz Biotechnology)
total RB (554136, BD Biosciences)
phospho RB Ser 780 (8180, Cell Signaling Technology)
E2F1 (sc-251, Santa Cruz Biotechnology)
E2F2 (ab138515, abcam)
E2F3 (PG37, Thermo FisherScientific)
E2F4 (WUF10, Thermo Fisher Scientific)
E2F5 (sc-999, Santa Cruz Biotechnology)
cyclin D1 (92G2, Cell Signaling Technology)
cyclin E2 (4132, Cell Signaling Technology)
CDK2 (78B2, Cell Signaling Technology)
GAPDH (14C10, Cell Signaling Technology)
actin (A2066, Sigma-Aldrich Chemie GmbH)
E1A (sc-25, Santa Cruz Biotechnology)
E1B55k (kindly provided by M. Dobbelstein)
E4orf6 (kindly provided by M. Dobbelstein
E2A (DBP, kindly provided by M. Dobbelstein)
Hexon (ABIN2686029, Antibodies online)

Small molecule inhibitor treatment

PD-0332991 isethionate (Palbociclib, Sigma-Aldrich Chemie GmbH) and LY-2835219 (Abemaciclib, Selleck Chemicals) were dissolved in sterile water as 10mM stock solution. LEE011 (Ribociclib, MedChem Express) and Nutlin-3a (Sigma) was dissolved in DMSO as 10mM and 5µM stock solution, respectively. Working concentrations were prepared freshly for immediate use.

Virus infection and combination treatment

For determination of virus induced cell killing, cells were seeded in 12-well plates. For combination treatment with PD-033299, LY-2835219, and LEE011, cells were pretreated with the inhibitors for 24 h. Cells were infected with the indicated viruses at indicated MOI in

200-400µl medium without FBS. At 1 hpi, complete medium with or without small molecule inhibitors was added to the cells.

Cell viability (SRB Assay)

Cells were fixed with 10% TCA for 1 h at 4°C and stained with 0.5% sulforhodamine B (SRB, Sigma-Aldrich Chemie GmbH) in 1% acetic acid for 30 min at RT, followed by washing with 1% acetic acid to remove excess of SRB. Dried SRB was dissolved in 10mM Tris buffer and quantification was performed by photometric measurement at 590nm.

Titer test

For determination of infectious viral particle production, infected cells and supernatant were harvested three dpi using cell scrapers. Virus was released from intact cells by multiple cycles of freeze-thaw followed by centrifugation at 1600 rcf. Supernatants of the cell lysates were tested for viral particle production using Hek293 cells as described in AdEasy Viral Titer Kit instruction manual (972500). The following reagents were used: goat-anti-hexon antibody (1056, Chemicon), rabbit-anti-goat antibody (P0449, Dako), DAB solution (Dako).

Example 2: Effect of CDK4/6 inhibitor PD0332991 on replication of an E1-minus adenovirus

It was shown that E1-deleted adenovirus replicates in cancer cells although with very low efficacy. T24 cells were infected with 100 MOI of an E1-minus adenovirus expressing green fluorescent protein (Ad-GFP), and treated with 500 nM PD0332991 one day before infection and during incubation time. Under such conditions, an increase in GFP expression was observed, thus indicating E1A-independent viral replication and gene expression mediated by the activation of the adenovirus E2-early promoter.

Example 3: Combined use of wild type adenovirus or XVir-N-31 together with different CDK4/6 inhibitors

Based upon results using the E2-early mutated adenovirus Ad-WT/E2M and Ad-GFP in combination with PD0332991, experiments were performed using different CDK4/6 inhibitors in combination with either wild type adenovirus Ad-WT or XVir-N-31. Since these agents arrest cells in phase G1, it was surprising to find that all inhibitors were able to support viral replication.

It was further examined if treatment of cells with the three clinically advanced CDK4/6 inhibitors PD-033299, LY-2835219 and LEE011 could influence the effects upon infection on cell viability, viral replication and viral titer production.

Upon treatment, all three inhibitors display similar effects on the expression and phosphorylation level of RB which has been described in numerous publications before. After an almost complete dephosphorylation and also downregulation of total protein at 24 hours, the phosphorylation level recovers partially over time. CDK2 level were upregulated upon treatment and cyclin D2 as well as cyclin E2 level were downregulated.

Example 4: Synergistic effects arising from the combination of CDK4/6 inhibitors and oncolytic adenoviruses

CDK4/6 inhibitors PD-033299, LY-2835219 and LEE011 were combined with the infection of cells with adenovirus. Infection of cells has been done 24 hours after treatment because downstream effects on target molecules can only be detected between 8 and 24 hours after treatment.

The results are shown in Fig. 1.

CDK4/6 inhibitors induced synergistic effects on cell viability, viral replication and viral titer. (a) Cells were pretreated with the three CDK4/6 inhibitors PD-033299, LY-2835219 and LEE011 for 24 hours and infected with XVir-N-31 (Moi 60) or wild type adenovirus (Moi 80). Four days past infection, cell viability was measured by an SRB assay. Graphs show averages of a minimum of three independent experiments. (b) Three days past infection, lysates were prepared from the cells and a titer test was performed on HEK293 cells. The virus titer is shown as fold change relative to control. (c) DNA was extracted from infected cells at 4, 24, 36 and 48 hpi and analysed for viral replication by using a qPCR for fiber cDNA. Values are normalized to GAPDH at 4 hpi. Graphs show representatives of at least two independent experiments. Error bars represent the standard error.

As evident from Fig. 1, all three CDK4/6 inhibitors dramatically supported cell lysis (Fig. 1a), the replication within cells (Fig 18) and the formation of viral particles (Fig 1b).

Example 5: Effect of CDK4/6 inhibitor palbociclib (PD-033299) on the expression level of selected viral proteins

In order to analyze these effects in greater detail, expression level of selected viral proteins was determined in treated or non-treated cells. For this experiment inhibitor palbociclib (PD-033299) was used as a representative CDK4/6 inhibitor. Cells were infected with a MOI of 15. PD treatment with 500 nM took place 24 hours infection and until protein isolation took place. After 12, 24 and 36 hours protein isolation took place using 1% SDS-buffer occurred. Actin was included as a positive control. Since the loading control shows same protein levels of cellular actin in all lines, a proper comparison between the lines is ensured. hpi: hours post infection

The results are indicated in Fig. 2 showing results of viral protein expression of Ad-WT and XVir-N-31 infected T24 cells in combination with the CDK4/6 inhibitor PD0332991 (PD). The viral proteins investigated in this experiment (E1A, E1B-55k, DBP (E2A) and Hexon) were all expressed at higher level in cells treated with the CDK4/6 inhibitor PD-0332991

compared to the adenovirus wild type virus. This effect could be observed as early as 12 hpi for E1A and 24 hpi for the other proteins.

Example 6: Specificity of effects mediated by CDK4/6 inhibitors

The class of CDK4/6 inhibitors as subject to Example 5 requires expression of RB. Therefor, three RB positive and two RB negative bladder cancer derived cell lines were used and the cells treated with the combination therapy. Cell lines were pretreated for 24 hours with an IC50 concentration of PD-0332991 (T24: 500 nM, RT112: 2000 nM, 253J: 100nM) and infected with XVir-N-31(T24 MOI50, 253J MOI 25, RT112 MOI450). Values are the average of at least 2 independent experiments. Error bars show the standard error. Four dpi, cell viability was measured using SRB assays (a, c). (b, d) Lysates of cells were prepared 3 dpi and a titer test was performed on Hek293 cells. Viral titer is shown as fold change relative to control

The results are shown in Fig. 3.

As evident from Fig. 3, only cell lines positive for RB showed a significant decrease in cell growth and cell viability, respectively (Fig. 3 a, c). Also, viral particle formation was only increased in RB positive cell lines upon PD-0332991 treatment (Fig. 3 b, d).

Example 7: Effect of combination treatment of CDK4/6 inhibitor PD-0332991 with XVir-N-31

In order to investigate the effect of PD-0332991 on viral replication in the RB positive cell lines, a relative quantification of Fiber DNA copies was performed using qPCR. Bladder cancer cell lines were pretreated for 24 hours and infected with XVir-N-31 (T24 MOI 40, UMUC3 and 253J MOI 20, RT112 MOI 400). DNA was extracted 24-48 hpi and analysed for viral fiber using qPCR. Values are normalized to GAPDH. Data are representatives of at least two independent experiments; Error bars S.D.

The results are shown in Fig. 4.

As may be taken form Fig. 4, combination treatment of CDK4/6 inhibitor PD-0332991 with XVir-N-31 increases viral replication dramatically.

Example 8: Time kinetics of CDK4/6 inhibitors

Time kinetics of CDK4/6 inhibitors on the dephosphorylation and degradation of RB are around 10 hours after treatment of cells. Also, the results presented above showed partial recovery of RB downstream targets over time (Figure 1). This observation implies that time kinetics of the CDK4/6 inhibitor and the effect on viral induced cell death is an important parameter for this combination therapy as exemplified in Example 7. For application of the combination therapy, different time points for pretreatment of cells were tested. In accordance therewith, cells were treated either before (day/hour ante infection, dai/hai) or 1 hour post infection and cell growth was measured using an SRB assay. Error bars represent S.E. and the values are the average of three independent experiments

The results are shown in Fig. 5

As evident from Fig. 5, parallel treatment already was sufficient for increase in cell death.

Example 9: Combination treatment of different adenoviruses with CDK4/6 inhibitor PD0332991

This example was performed so as to provide experimental evidence that different oncolytic adenoviruses may be used together with CDK4/6 inhibitors such as PD0332991 for cell killing, and that the observed increase in viral replication and cell killing was not restricted to XVir-N-31. In accordance therewith, T24 cancer cells with Ad-Delta24 and Onyx-015 as follows: T24 bladder cancer cells were infected with 20 MOI of the indicated oncolytic adenoviruses. Treatment with 500 nM CDK4/6 inhibitor PD0332991 took place one day

before infection and for 4 days post infection. Pictures were taken 4 days post infection. The occurrence of cytopathic effect (CPE) indicates viral replication and cell killing.

The results are shown in Fig. 6.

As may be taken from Fig. 6, CDK4/6 inhibitor PD0332991 as a representative example of CDK4/6 inhibitors reducing RB phosphorylation, increased cell killing when combined with other oncolytic adenoviruses such as Ad-Delta24 and Onyx-015.

Example 10: Infection of T24 cells with the recombinant E1-deleted adenovirus expressing GFP (Ad-minus/GFP) in combination with Palbociclib causes increase GFP expression

100.000 T24 cells/well were seeded in 6-well plates and grown in RPMI Medium containing 10 %FCS at 5% CO_2 at 37°C. T24 cells were treated with 500 nM Palbociclib 24 hours before and again 1 hour post infection. Infection of the E1-deleted adenovirus expressing GFP (Ad-minus/GFP) took place in 400 µl Medium without serum. Pictures were taken 48 hours post infection using a fluorescence microscope with 10x magnification.

The result of fluorescence microscopic analysis of GFP expression with and without Palbociclib treatment is shown in Fig. 7.

The result shows that treatment of T24 cells with Palbociclib caused a strong increase of GFP expression which is mediated by viral DNA replication induced by Palbociclib.

Example 11: E1A-independent viral replication in UMUC cells treated with various cell cycle inhibitors

To investigate the differences in replication of dl703 (Mantwill et al. 2013, Journal of Translational Medicine, 11, 216) under different treatment conditions, DNA-replication analysis was performed. 100.000 UMUC cells were seeded in 6-well plates and grown in

DMEM medium containing 10%FCS in 5% CO_2 conditions at 37°C. 24 hours post seeding cells were treated for 24 hours with 10 µM Lee (Ribociclib), 1 µM CI-1040, 10 µM Nutlin-3a and 10 µM Roscovertine and again after infection adding an appropriate amount of inhibitors to the medium. Infection with 50 MOI dl703 (Mastadenovirus, Type C, Serotype 5 with a 3.2 kb sized deletion in E1 region) took place 24 hours post treatment. After 4 and 48 hours post infection DNA were isolated and qPCR was performed using specific primers for the viral fiber gene. Fiber fwd. 5`-AAGCTAGCCCTGCAAACATCA-3` (SEQ ID NO: 17); Fiber rev. 5`-CCCAAGCTACCAGTGGCAGTA-3` (SEQ ID NO: 18).

The result is shown in Fig. 8.

As evident from Fig. 8, treatment of UMUC cells with the CDK 4/6 inhibitor LEE011 (Ribociclib) caused a dramatic increase of viral DNA replication of the E1-minus adenovirus dl703 (nearly 100-fold). This increase strongly suggests that the specific induced G1-arrest by Ribociclib in conjunction with the inhibition of E2F1 expression facilitates E1-independend adenoviral replication. In consequence, not only viruses with specific deletions in the E1A gene show enhanced adenovirus DNA replication under CDK 4/6 treatment, but even adenoviruses with complete deletion of the E1A gene show an increase in viral DNA replication.

Although the Mek-Inhibitor GI-1040 showed similar properties regarding inhibition of E2F1 expression and G1-arrest, the replication was much lower compared to Ribociclib treated cells. This might be due to the fact that simultaneously other important cell cycle related pathways are inhibited such as MEK/ERK which is necessary for viral replication. In addition, it was shown that inhibition of the MEK/ERK-Pathway reduced particle formation more than 100-fold making it unsuitable, in a clinical setting, for combination therapy with oncolytic adenovirus replication (Schümann and Doppelstein 2016, Cancer Research, 66, 1282-1288).

Example 12: Western Blot Analysis of UMUC cells treated with indicated cell cycles inhibitors

Western Blot analysis of UMUC cells treated with indicated concentrations of CI-1040, Roscovitine, Nutlin-3a and LEE011 (Ribociclib). 1 x 106 cells were seeded in 10 cm dishes. 24 hours post-treatment proteins were isolated using 1% SDS buffer, to achieve the disruption of the nuclear membrane. All samples were drawn up several times into a syringe to disrupt the DNA and subsequently centrifuged at 30000 rpm at 4 °C for 30 minutes. The supernatant was transferred to a new reaction tube and directly used for further steps or stored at -80 °C. To separate the proteins a sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed. By means of electrophoresis for approximately two hours at 100V at 4°C, 40 µg of total proteins were loaded and probed against specific indicated antibodies.

The results are shown in Figs. 9A, 9B and 9C.

As evident from Fig. 9, whereas Roscovitine and Nutlin-3a had no pronounced effect on Rb, phRB and E2F1 expression, LEE-011 (Ribociclib) at 10 µM and MI-1040 at 1 µM induced inhibition of E2F1 as well as Rb and phRb expression.

Example 13: Analysis of CDK 4/6 inhibitors on viral DNA replication of the E1-deleted replication defective adenovirus dl703

For cell cycle analysis cells were seeded in 6 well plates (2,5x10E4 c/well). 8 hours before infection with dl703, cells were treated with indicated concentration of cell cycle inhibitors. After infection with 10 MOI dl703 cells were again treated for 48 hours. Untreated cells and dl703 infected cells only, served as control. 48h post infection cells were harvest by trypsinization and fixed with 80 % ethanol while vortexing. To investigate the cell cycle status, fixed cells were centrifuged 5 min at RT and 300g and ethanol was aspirated. Cells were resuspended and washed with 1%BSA-PBS (Bovine Serum Albumin) and again centrifuged. Cells were stained with EDU and cell cycle analysis were performed using the Click-iT™ Plus EdU Flow Cytometry Assay Kits, Catalog nos. C10632 from Thermo

Fischer. In addition, after 3x times washing with 1% BSA/PBS cells were stained with PI (Propidium Iodine,50 µg/ml). Measurement was directly performed after staining with a FACScalibur Flow Cytometry System. Data was analyzed with FlowJo software.

Characteristics of the CDK4/6 inhibitors

CI1040: The dual specific threonine/tyrosine kinase, map kinase kinase (MEK), is a key component of the RAS/RAF/MEK/ERK signaling pathway that is frequently activated in human tumors. CI-1040 is a benzhydroxamate compound that potently inhibits MEK (Allen et al. 2003, Semin Oncol. (5 Suppl 16):105-16) and causes G1 arrest.

Nutlin-3a: Nutlin-3, a small-molecule antagonist of MDM2, effectively restores p53 function in both normal MDM2 expression and MDM2 overexpression cell lines with wild-type p53, leading to cell cycle arrest and apoptosis (Wang et al 2012, Acta Biochimica et Biophysica Sinica, Volume 44, Issue 8, 1 August 2012, Pages 685–691).

Roscovitine (Seliciclib or CYC202) is an experimental drug candidate in the family of pharmacological cyclin-dependent kinase (CDK) inhibitors that preferentially inhibit multiple enzyme targets including CDK2, CDK7 and CDK9, which alter the growth phase or state within the cell cycle of treated cells (Whitaker et al. 2004, Cancer Research 64, 262–272).

LEE011 (Ribociclib; trade name Kisqali]) is an inhibitor of cyclin D1/CDK4 and CDK6, and is used for the treatment of certain kinds of breast cancer. The inhibition of CDK 4/6 causes G1 cell cycle arrest and inhibition of E2F1 expression (Kim S. et al, Oncotarget. 2018 Oct 16;9(81):35226-35240; Yang C et al., Oncogene (2017)36,2255–2264).

The results are shown in Fig. 10.

The CDK 4/6 inhibitors LEE011 (Ribociclib) and CI-1040 induced a clear G1-arrest. Treatment with Roscovitine showed a slight increase of G2/m arrested cells. Nutlin-3a had only little or no effect on the cell cycle in the used concentration. Infection of UMUC cells with the recombinant E1-deleted (having no E1A protein) adenovirus dl703 did not change the cell cycle distribution significantly.

Example 14: Palbociclib increased adenovirus hexon staining in vitro post treatment

Bladder cell lines RT112, T24 and UMUC were seeded in 6-well plates (2 x105 cells/well). One day post seeding cells were treated with 500 nM Palbociclib for 24 hours before and again 1 hour post infection. Infection with indicated MOIs of AD-WT took place in 400 µl DMEM-Medium without serum. Hexon staining was performed according manufacturer instructions using Adeasy Viral Titer Kit from Agilent (cat: 972500) two days post infection.

The result is shown in Fig. 11.

As evident from Fig. 11, treatment of Palbociclib (500 nM) as an exemplary CDK4/6 inhibitor increased hexon positive cells significantly in Palbociclib treated cells 48 hours post infection as indicated by the brown/red colour. The conclusion must be reached, that more cells under Palbociclib treatment are capable to produce viral particles and show increase viral DNA replication, since adenovirus hexon expression occurs exclusive onset of viral replication.

From the results subject to Examples 10 to 14, it is evident that only CDK4/6 inhibitors, but no other cell cycle inhibitors were capable of increasing replication and gene expression of replication defective adenovirus (dl703 lacking the E1 genes) and Ad-GFP. Furthermore, a CDK4/6 inhibitor in order to provide such increased viral replication and gene expression must cause G1 arrest of (infected) cells and inhibition of F2F1 expression.

Example 15: Treatment of T24 cells using triple therapy comprising XVir-N-31, Palbociclib and a PARP inhibitor

In order to show the efficacy of a triple therapy of T24 cells using triple therapy comprising XVir-N-31, Palbociclib and a PARP inhibitor (BMN673 (Talazolarib)), a potency assay was carried out.

12.500 T24 cells were seeded per well in 12-well plates and grown over-night in RPMI Medium containing 10 % FCS at 37°C. Inhibitor-treatment of cells occurred 24 h past cell seeding and again 1 hour after infection by adding indicated concentration to the medium. Infection of cells took place 24 h past inhibitor-treatment in 250 µl medium without serum. Fixation and SRB-staining took place at 4 days post infection. PD, Palbociclib; PARPi: BMN673.

For SRB staining, the medium was removed by aspiration. Cells were fixed with 1 ml (per well) 10 % cold TCA at 4°C for 1 hour. TCA was removed by aspiration and cell layers were washed 4 × with tap water. Cells were stained with 1 ml (per well) 0.5% SRB (sulforhodamine B) in 1% acetic acid for 30 min. Unbound SRB was removed in five washing steps with 1 ml 1% acetic acid /well; after each washing step, acetic acid was removed by aspiration. Plates were air-dried for 2 hrs. To solubilize the SRB stained cells, 200 µl of 10 mM Tris base was added to each well. Afterwards 20 µl, respectively, was dispensed into wells of a 96 well plate. The 96 well plate was loaded into an Elisa-plate reader and absorption of the samples was measured at 560 nm. Mock treated cells were set 100 % cell survival.

The result is shown in Fig. 12.

The result shown in figure 12 clearly demonstrates that the triple therapy consisting of Palbociclib, BMN673 and XVir-N-31 exhibited a superior performance against mono- or combination therapy regarding cell killing. Nearly 90% cell killing could be achieved using 10 MOI of XVir-N-31 in combination with PARP inhibitor PARPi (BMN673) and CDK4/6 inhibitor Palbociclib (PD). The combination of PARPi and Palbociclib without XVir-N-31

killed only 65% of the cells. T24 cells and UMUC cells are sensitive to CDK 4/6-Inhibitors (providing G1 arrest with E2F1 down-regulation).

Example 16: Kinetics of triple therapy comprising XVir-N-31, Palbociclib and a PARP inhibitor

In order to show the kinetics of a triple therapy of T24 cells using triple therapy comprising XVir-N-31, Palbociclib and a PARP inhibitor (BMN673 (Talazolarib)), a potency assay was carried out and the potency assessed at different points in time.

3000 T24 cells were seeded per well in 12-well plates and grown over-night in RPMI Medium containing 10 %FCS at 37°C. Inhibitor-treatment of cells occurred 24 h past cell seeding and again 1 hour after infection by adding indicated concentration to the medium. Infection of cells took place 24 h past inhibitor-treatment in 250 µl Medium without serum. Fixation and SRB-staining took place at 1-5 days post infection (dpi: days post infection). 15 nM PARPi correspond to the IC-80 value in T24 cells.

The results are shown Fig. 13.

As evident from Fig. 13, triple therapy using apart from XVir-N-31 a CDK4/6 inhibitor (Palbociclib (PD) and a PARP inhibitor PARPI (BMN673) is, also from a kinetic point of view, much more effective than a monotherapy using XVir-N-31 only or a combination therapy using XVir-N-31 an either the PARP inhibitor or the CDK4/6 inhibitor. Importantly, the re-growth of tumor cells was significantly reduced at day 4 and 5 in the CDK 4/6 sensitive cell lines UMUC and T24 (dpi: days post infection).

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 106

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 106

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

Claims

1. A combination comprising an adenovirus, and a CDK4/inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor, nutlin or a derivative of nutlin.

2. The combination of claim 1, wherein the combination further comprises a PARP inhibitor.

3. The combination of any one of claims 1 and 2, wherein the combination further comprises a bromodomain inhibitor.

4. The combination of any one of claims 1 to 3, wherein the combination further comprises nutlin or a nutlin derivative.

5. The combination of any one of claims 1 to 4 for use in a method for the treatment of a tumor or cancer.

6. An adenovirus for use in a method for the treatment of a tumor or cancer in a subject, wherein the method comprises administering to the subject the adenovirus, a CDK4/6 inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor, nutlin or a derivative of nutlin.

7. A CDK4/6 inhibitor for use in a method for the treatment of a tumor or cancer in a subject, wherein the method comprises administering to the subject an adenovirus, the CDK4/6 inhibitor and at least one further agent selected from the group comprising a PARP inhibitor, a bromodomain inhibitor, nutlin or a derivative of nutlin.

8. The combination of claim 1, the combination for use of claim 5, the adenovirus for use of claim 6 and the CDK4/6 inhibitor for use of claim 7, wherein the adenovirus is an oncolytic adenovirus.

9. The combination of any one of claims 1 and 8, the combination for use of any one of claims 5 and 8, the adenovirus for use of any one of claims 6 and 8, and the CDK4/6 inhibitor for use of any one of claims 7 and 8, wherein the adenovirus is selected from the group comprising XVir-N-31, dl520, AdΔ24, AdΔ24-RGD, dl922-947, E1Ad/01/07, dl1119/1131, CB 016, VCN-01, E1Adl1107, E1Adl1101, ORCA-010, Enadenotucirev and viruses lacking an expressed viral oncogene which is capable of binding a functional Rb tumor suppressor gene product.

10. The combination of any one of claims 1 and 8 to 9, the combination for use of any one of claims 5 and 8 to 9, the adenovirus for use of any one of claims 6 and 8 and 9, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 9, wherein the adenovirus is XVir-N-31.

11. The combination of any one of claims 1 and 8 to 10, the combination for use of any one of claims 5 and 8 to 10, the adenovirus for use of any one of claims 6 and 8 to 10, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 10, wherein the CDK4/6 inhibitor is a CDK4/6 inhibitor arresting cells in the G1 phase and inhibiting E2F1.

12. The combination of any one of claims 1 and 8 to 11, the combination for use of any one of claims 5 and 8 to 11, the adenovirus for use of any one of claims 6 and 8 to 11, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 11, wherein the CDK4/6 inhibitor is selected from the group comprising palbociclib which is also referred to as PD 0332991, abemaciclib which is also referred to as LY-2835219, ribociclib which is also referred to as LEE011, Trilaciclib which is also referred to as G1T28, and Dinaciclib.

13. The combination for use of any one of claims 5 and 8 to 12, the adenovirus for use of any one of claims 6 and 8 to 12, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 12, wherein the disease tumor or cancer is Rb-negative.

14. The combination for use of any one of claims 5 and 8 to 12, the adenovirus for use of any one of claims 6 and 8 to 12, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 12, wherein the disease tumor or cancer is expressing Rb or is Rb-positive.

15. The combination for use of any one of claims 5 and 8 to 14, the adenovirus for use of any one of claims 6 and 8 to 14, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 14, wherein the cells of the tumor cells have a resistance to or are insensitive to one or several pharmaceutically active agents and/or radiation.

16. The combination for use of any one of claims 5 and 8 to 14, the adenovirus for use of any one of claims 6 and 8 to 14, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 15, wherein the tumor or cancer contains YB-1 in the cell nucleus independent of the cell cycle.

17. The combination for use of any one of claims 5 and 8 to 15, the adenovirus for use of any one of claims 6 and 8 to 16, and the CDK4/6 inhibitor for use of any one of claims 7 and 8 to 16, wherein the disease is selected from the group comprising bladder cancer, breast cancer, metastatic breast cancer (mBC), melanoma, glioma, pancreatic cancer, hepatocellular carcinoma, lung adenocarcinoma, sarcoma, ovarian cancer, renal cancer, prostate cancer, and leukemia.

Fig. 1

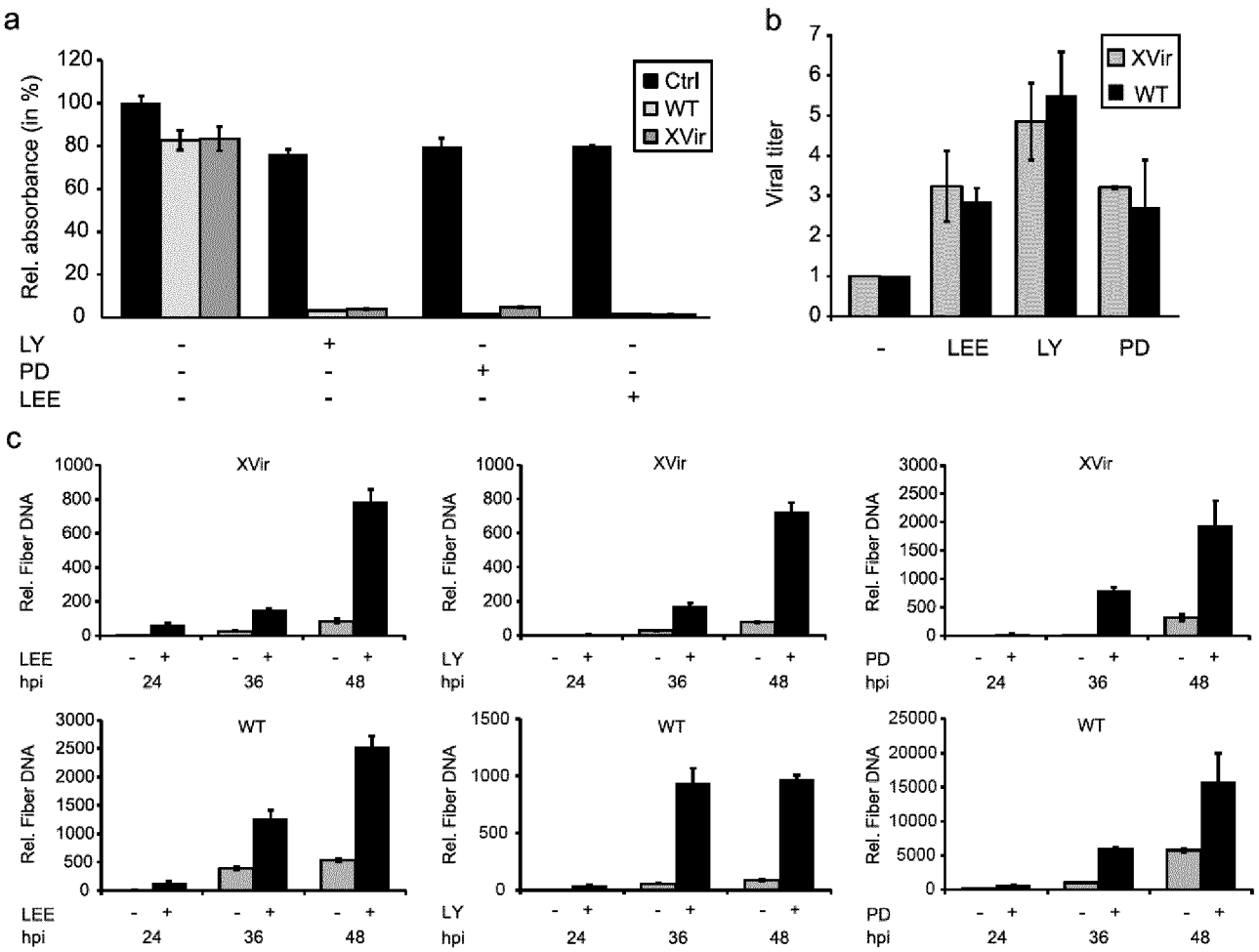

a
Rel. absorbance (in %)
120
100
80
60
40
20
0
Ctrl
WT
XVir
LY - + - -
PD - - + -
LEE - - - +
b
Viral titer
7
6
5
4
3
2
1
0
XVir
WT
- LEE LY PD
c
Rel. Fiber DNA
XVir
WT
LEE
LY
PD
hpi
24
36
48

Fig. 2

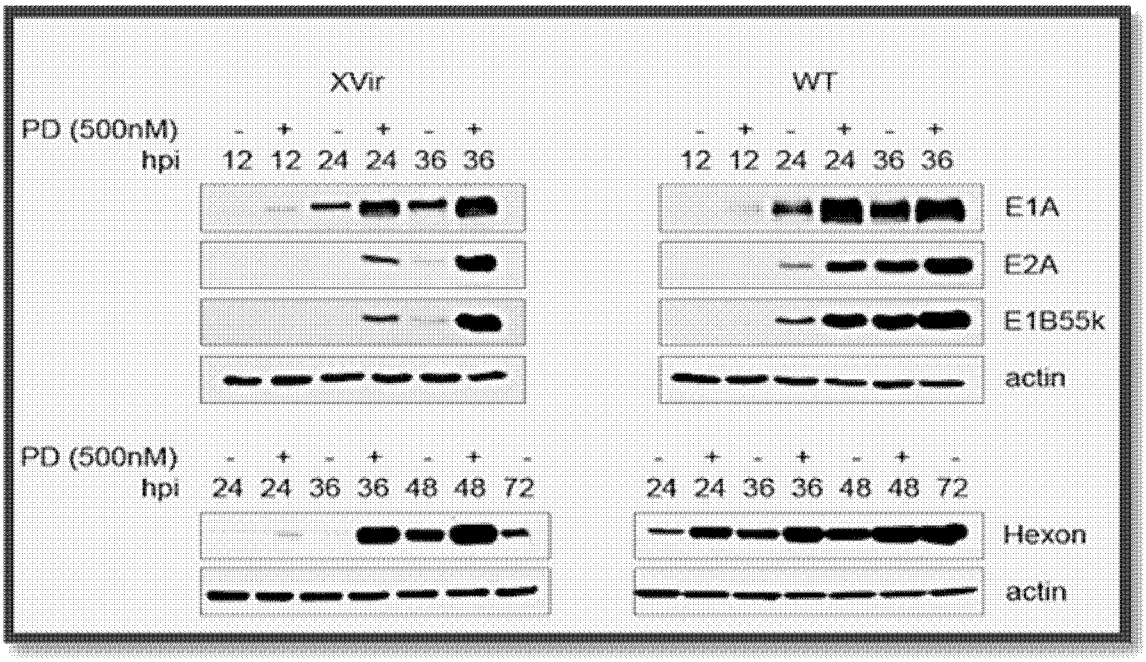

XVir
WT
PD (500nM)
hpi
12 12 24 24 36 36
E1A
E2A
E1B55k
actin
PD (500nM)
hpi
24 24 36 36 48 48 72
Hexon
actin

Fig. 3

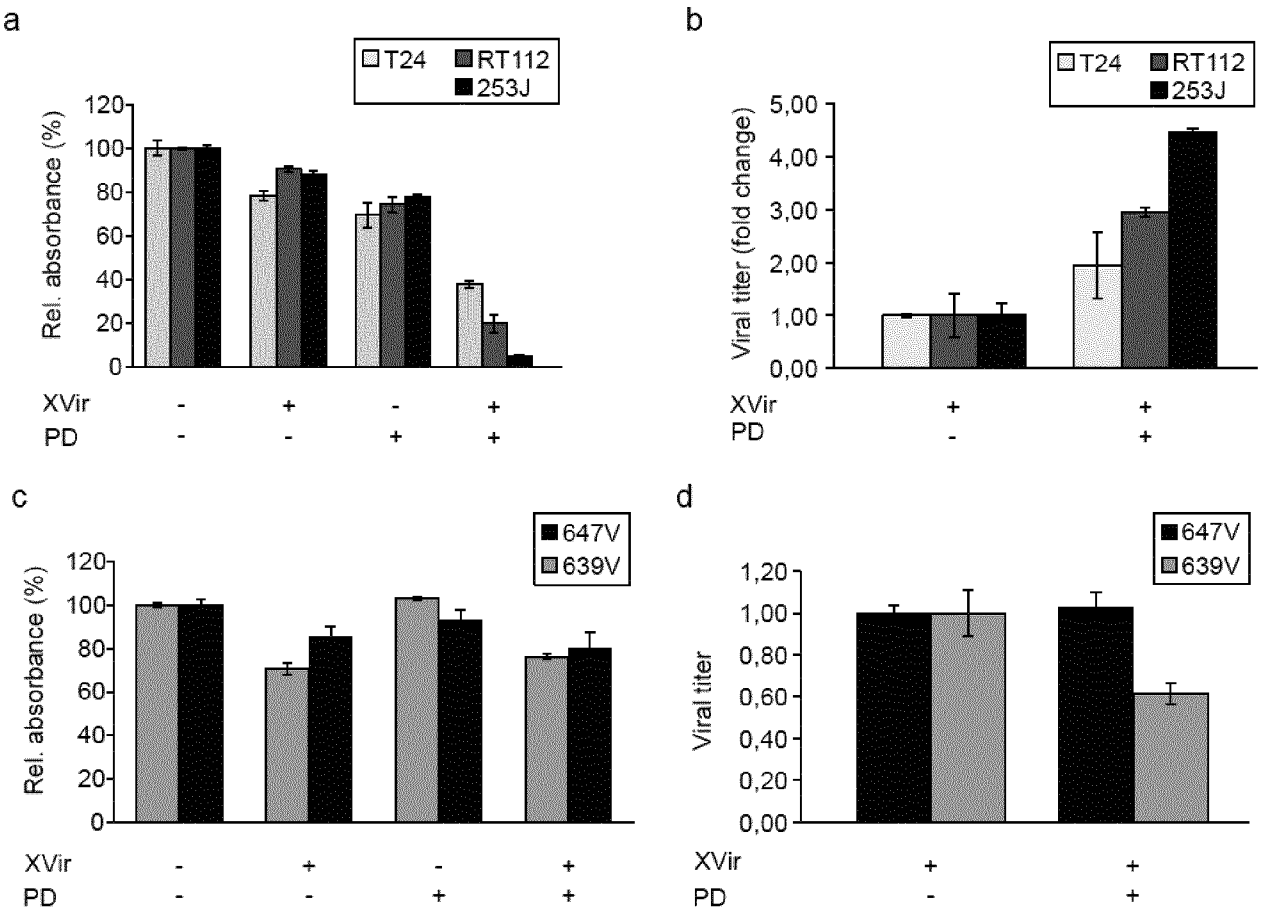

a
T24
RT112
253J
Rel. absorbance (%)
120
100
80
60
40
20
0
XVir - + - +
PD - - + +
b
T24
RT112
253J
Viral titer (fold change)
5,00
4,00
3,00
2,00
1,00
0,00
XVir + +
PD - +
c
647V
639V
Rel. absorbance (%)
120
100
80
60
40
20
0
XVir - + - +
PD - - + +
d
647V
639V
Viral titer
1,20
1,00
0,80
0,60
0,40
0,20
0,00
XVir + +
PD - +

Fig. 4

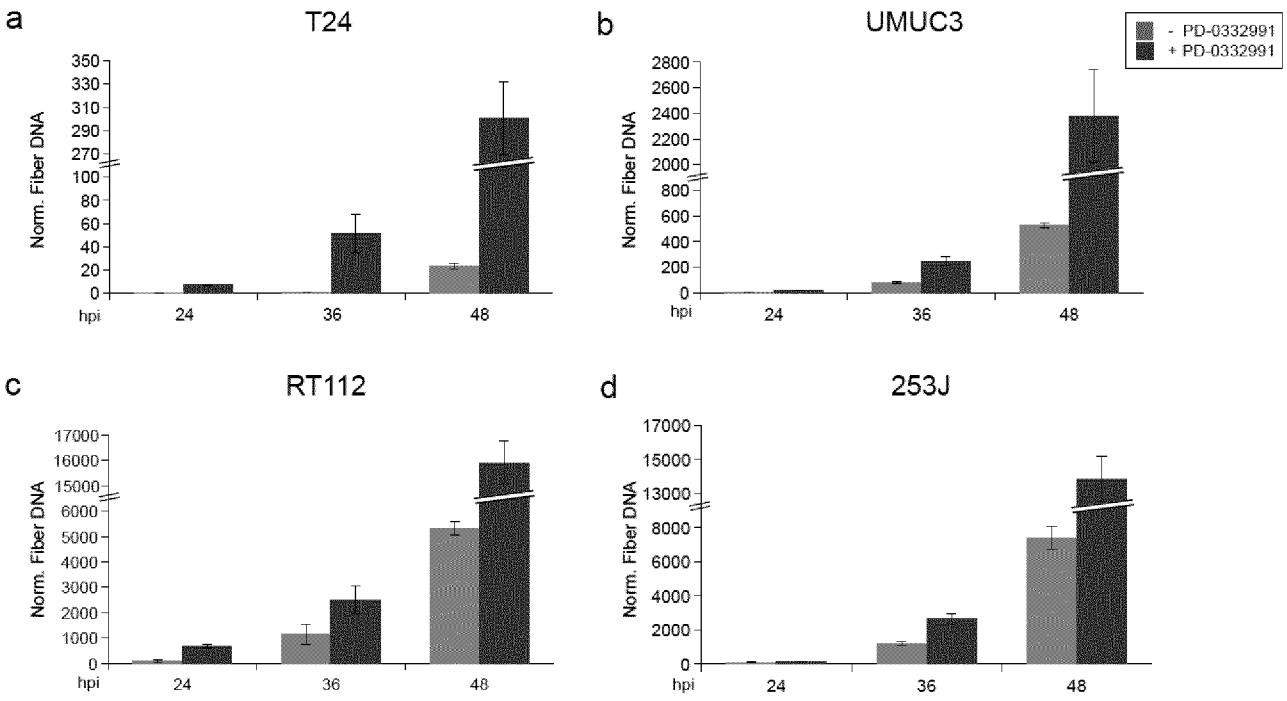
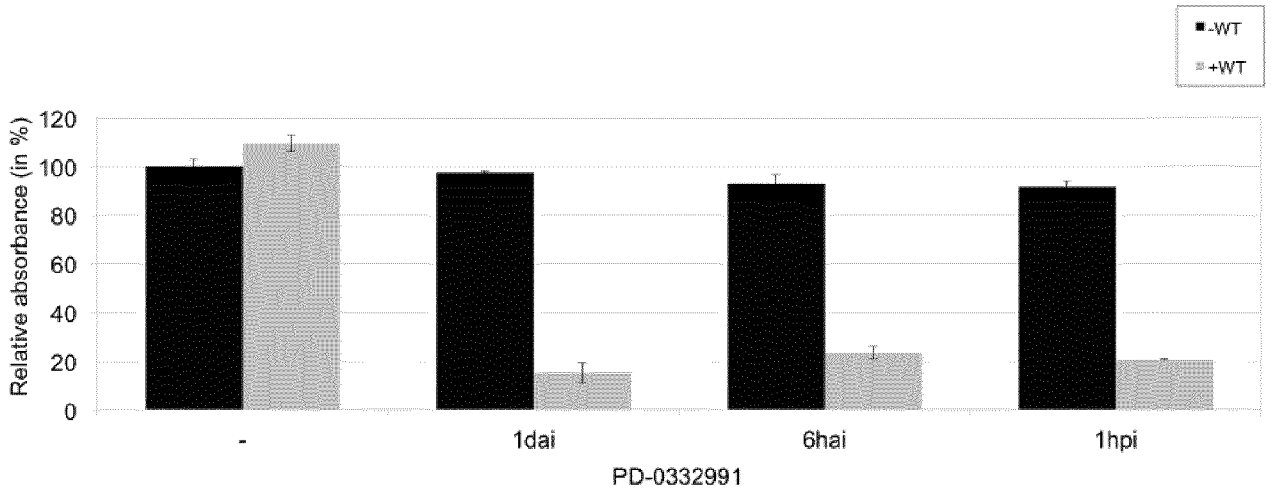

a
T24
b
UMUC3
- PD-0332991
+ PD-0332991
Norm. Fiber DNA
hpi
24
36
48
c
RT112
d
253J

Fig. 5

a


-WT
+WT
Relative absorbance (in %)
120
100
80
60
40
20
0
-
1dai
6hai
1hpi
PD-0332991

b

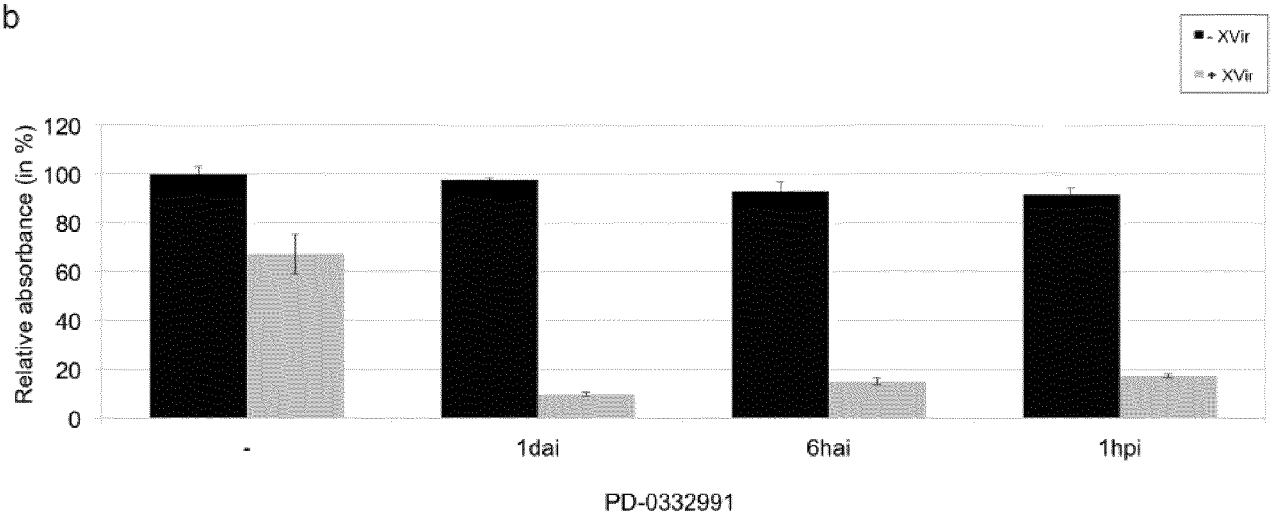

- XVir
+ XVir
Relative absorbance (in %)
120
100
80
60
40
20
0
-
1dai
6hai
1hpi
PD-0332991

Fig. 6

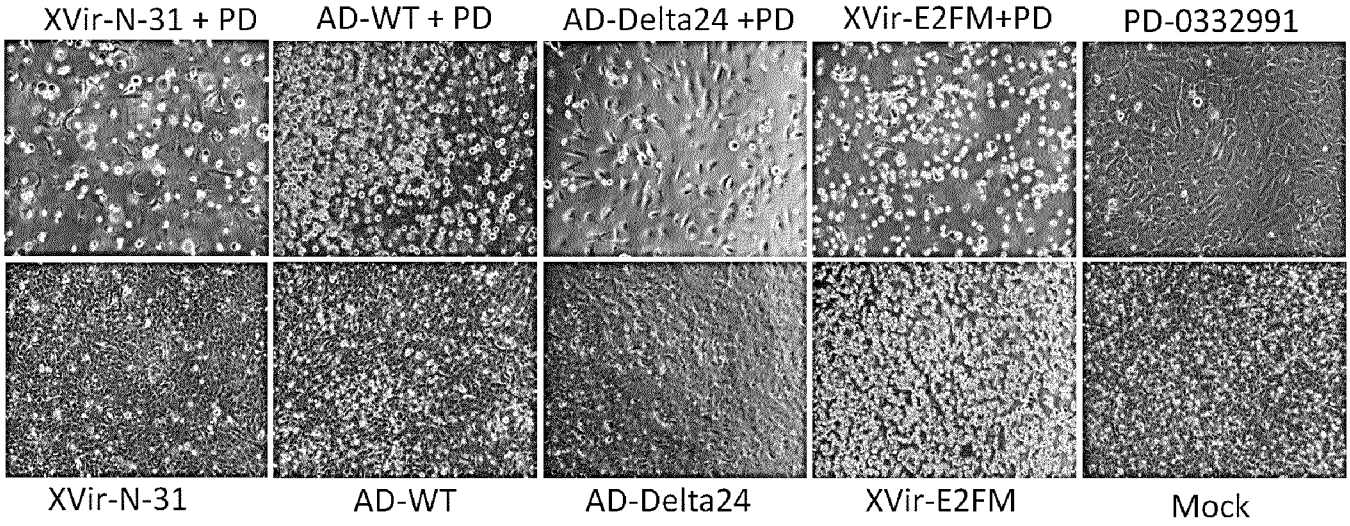

XVir-N-31 + PD
AD-WT + PD
AD-Delta24 +PD
XVir-E2FM+PD
PD-0332991
XVir-N-31
AD-WT
AD-Delta24
XVir-E2FM
Mock

Fig. 7

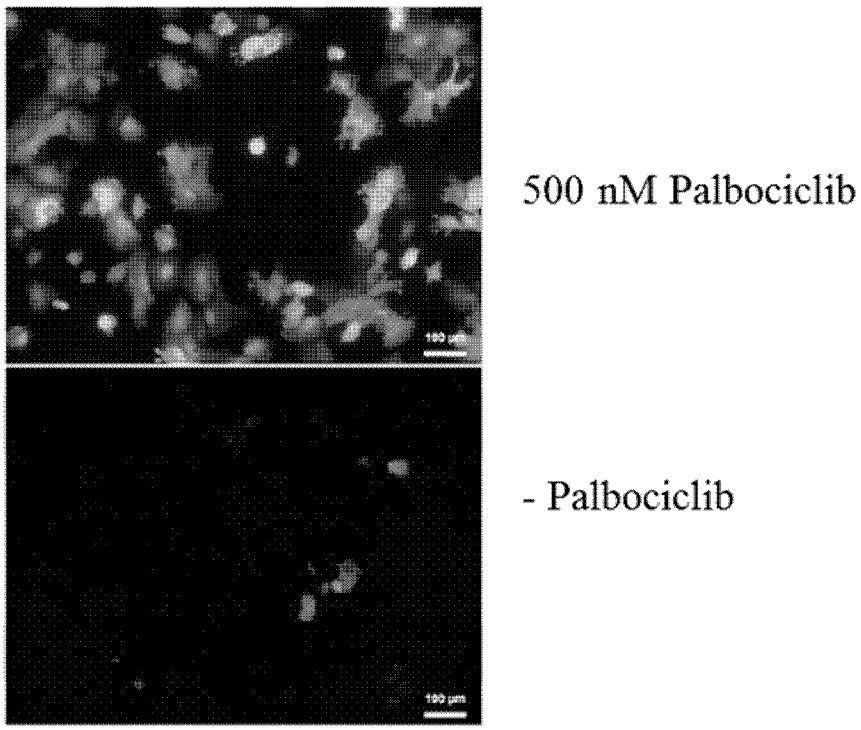

500 nM Palbociclib
- Palbociclib

Fig. 8

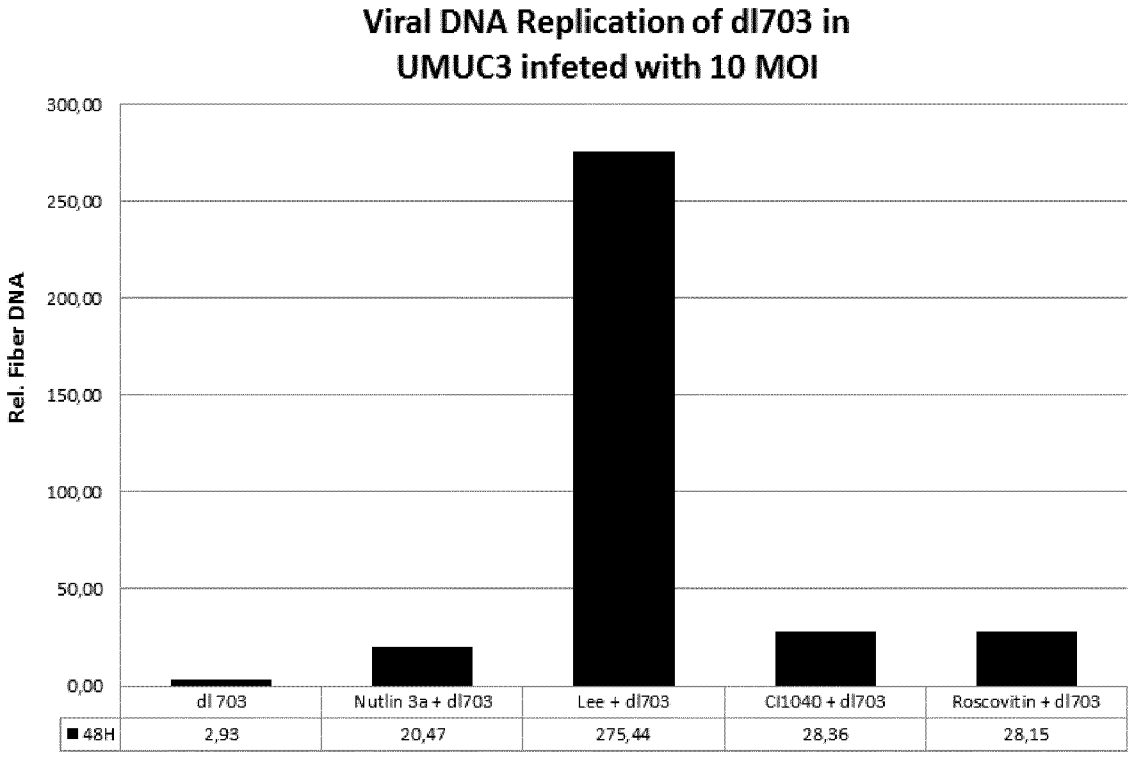

Viral DNA Replication of dl703 in
UMUC3 infeted with 10 MOI
Rel. Fiber DNA
300,00
250,00
200,00
150,00
100,00
50,00
0,00
dl 703
Nutlin 3a + dl703
Lee + dl703
CI1040 + dl703
Roscovitin + dl703
48H
2,93
20,47
275,44
28,36
28,15

Fig. 9A

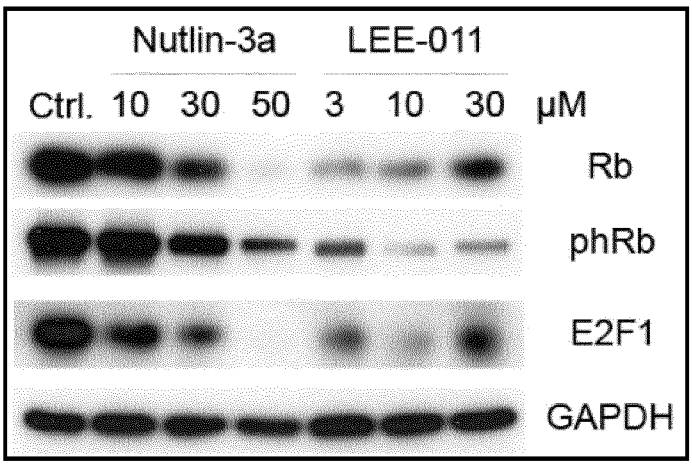


Fig. 9B

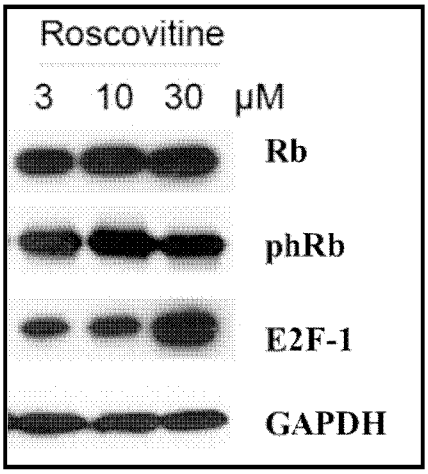


Fig. 9C

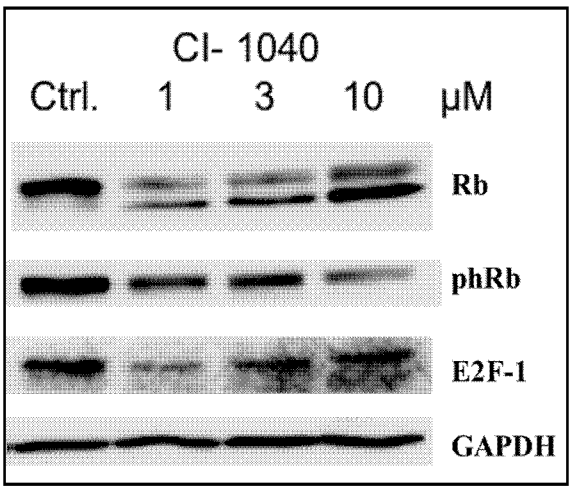


Fig. 10

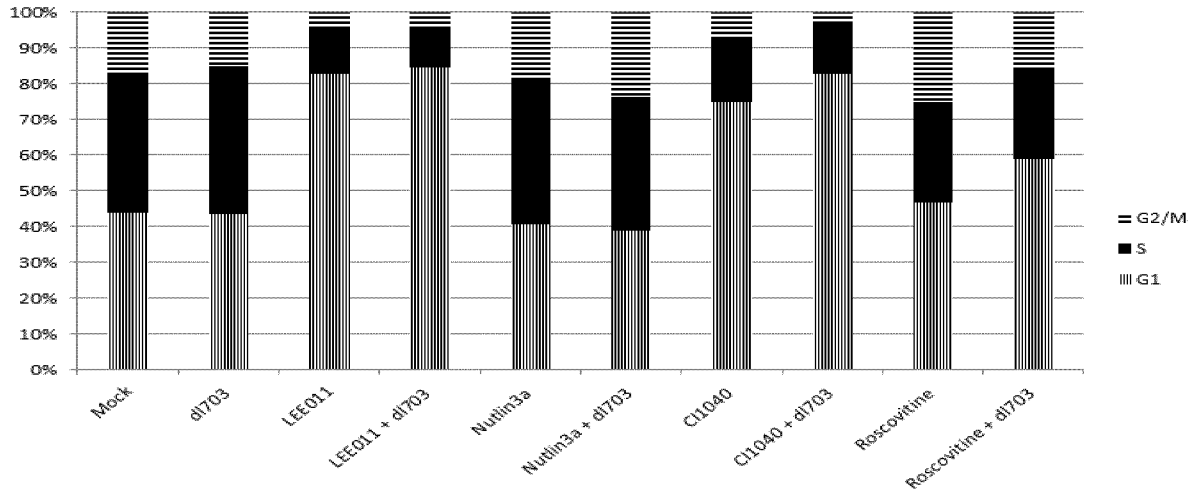

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Mock
dl703
LEE011
LEE011 + dl703
Nutlin3a
Nutlin3a + dl703
CI1040
CI1040 + dl703
Roscovitine
Roscovitine + dl703
G2/M
S
G1

Fig. 11

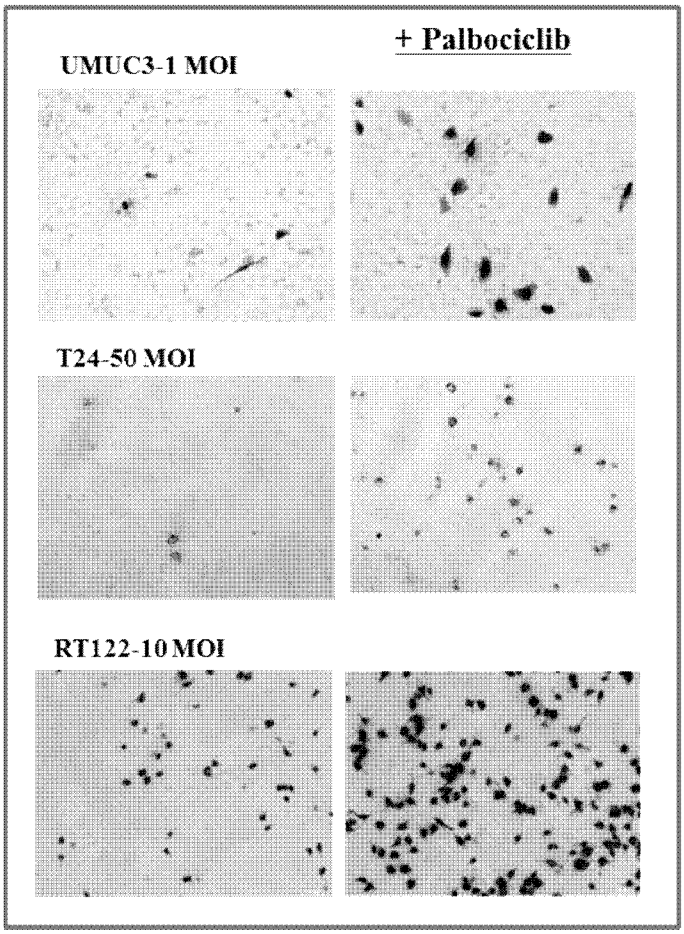

+ Palbociclib
UMUC3-1 MOI
T24-50 MOI
RT122-10 MOI

Fig. 12

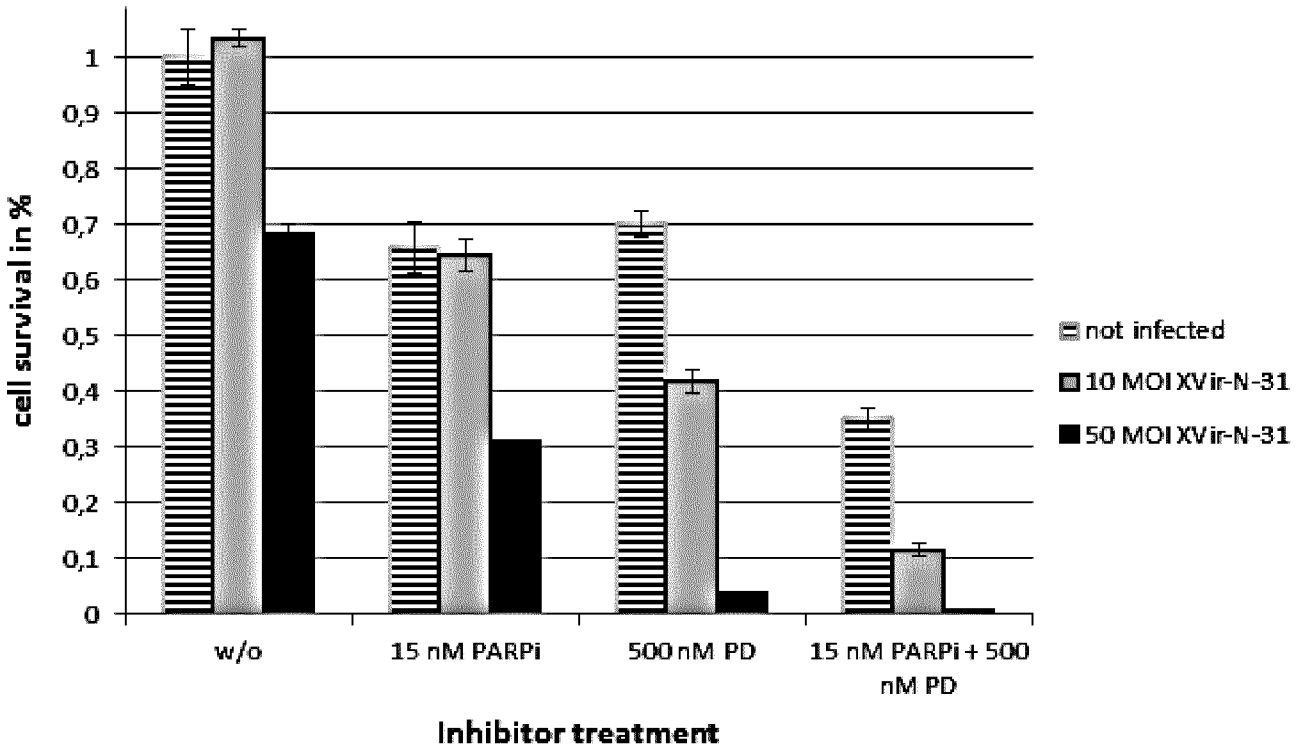
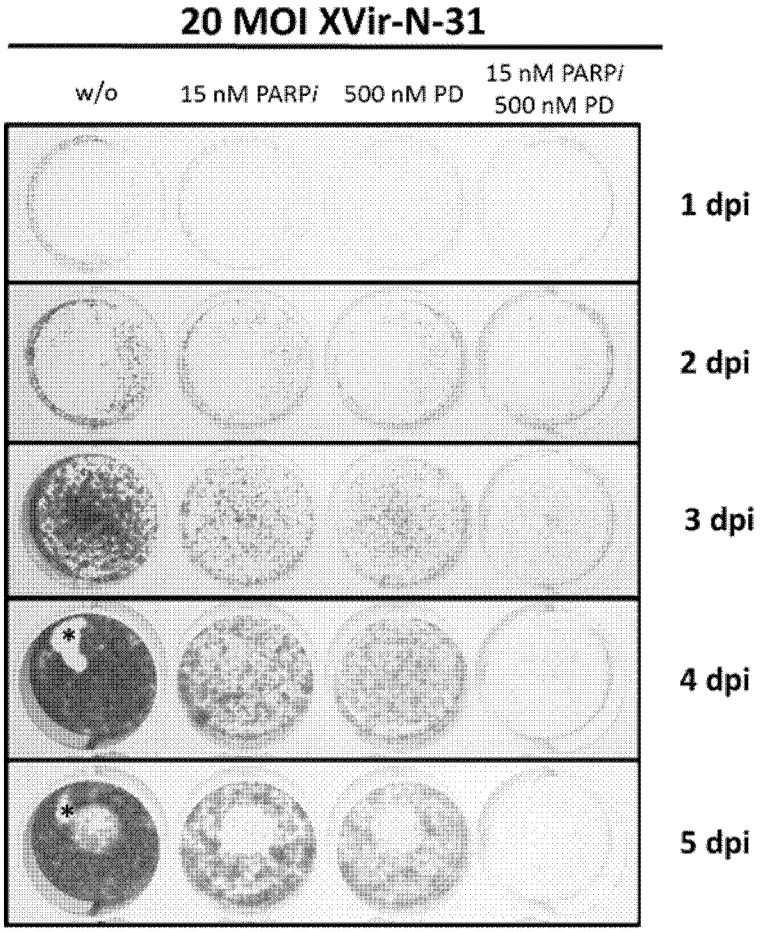

cell survival in %
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
w/o
15 nM PARPi
500 nM PD
15 nM PARPi + 500 nM PD
Inhibitor treatment
not infected
10 MOI XVir-N-31
50 MOI XVir-N-31

Fig. 13



Asterisks (*) indicate artefacts from cell fixing

Fig. 14

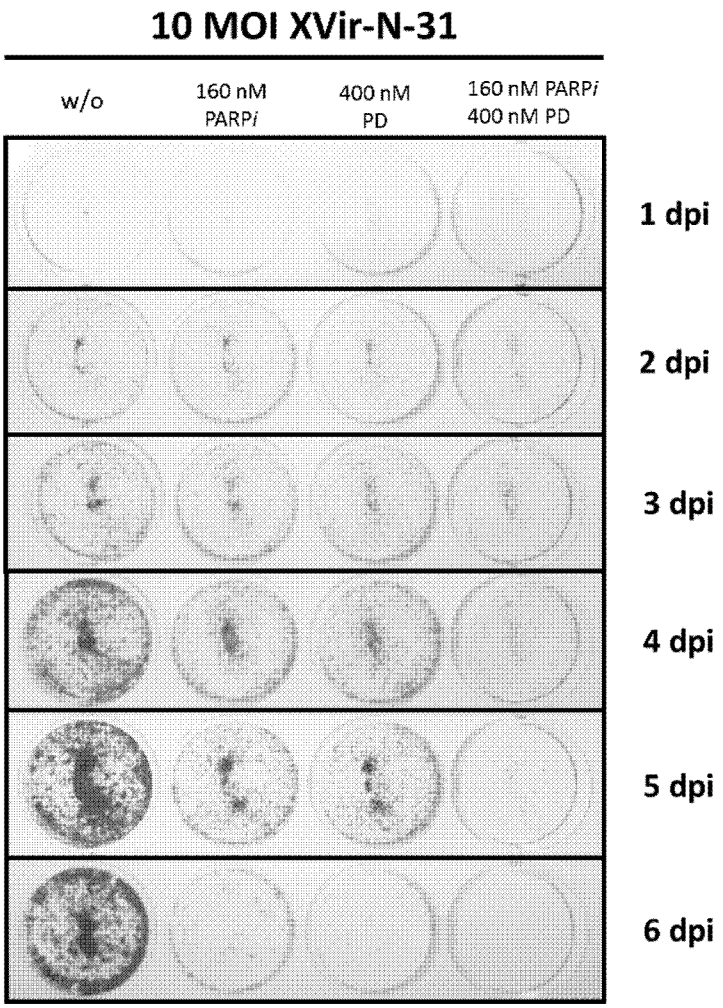

10 MOI XVir-N-31
w/o
160 nM PARPi
400 nM PD
160 nM PARPi 400 nM PD
1 dpi
2 dpi
3 dpi
4 dpi
5 dpi
6 dpi

Fig. 15

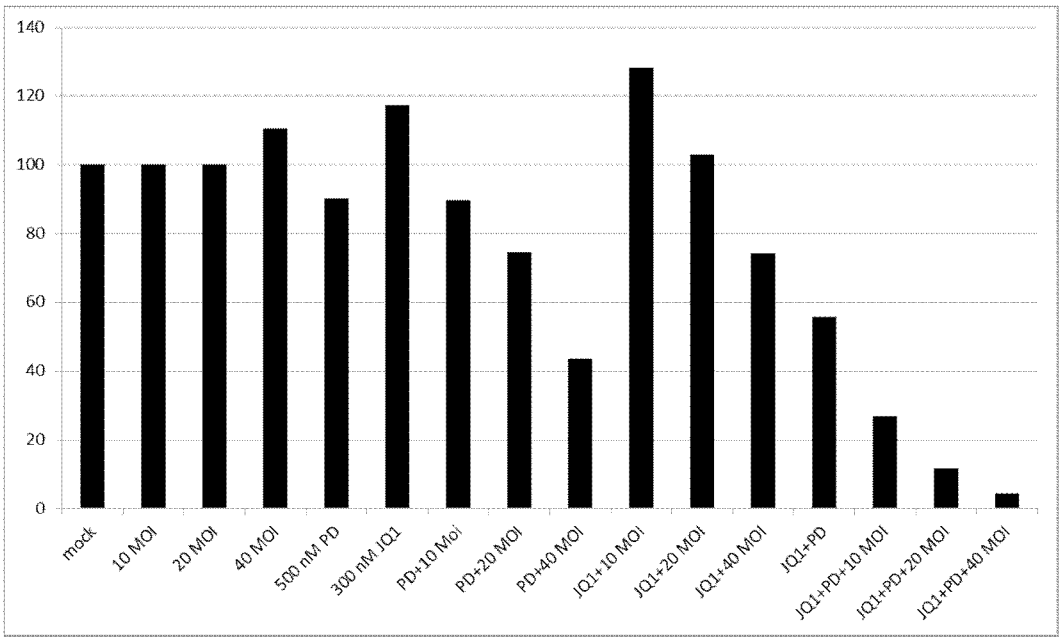

140
120
100
80
60
40
20
0
mock
10 MOI
20 MOI
40 MOI
500 nM PD
300 nM JQ1
PD+10 Moi
PD+20 MOI
PD+40 MOI
JQ1+10 MOI
JQ1+20 MOI
JQ1+40 MOI
JQ1+PD
JQ1+PD+10 MOI
JQ1+PD+20 MOI
JQ1+PD+40 MOI

Fig. 16

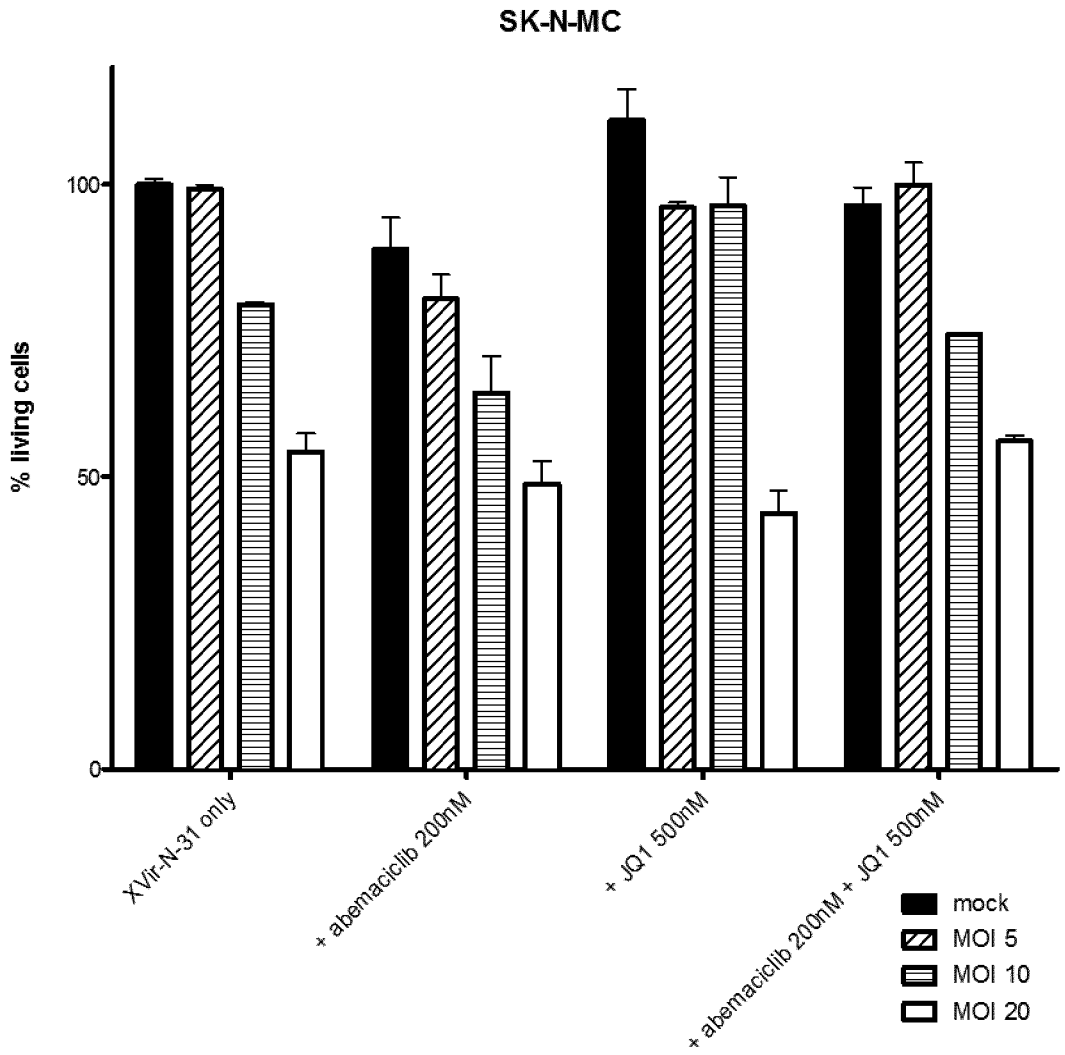

SK-N-MC
% living cells
100
50
0
XVir-N-31 only
+ abemaciclib 200nM
+ JQ1 500nM
+ abemaciclib 200nM + JQ1 500nM
mock
MOI 5
MOI 10
MOI 20

Fig. 17

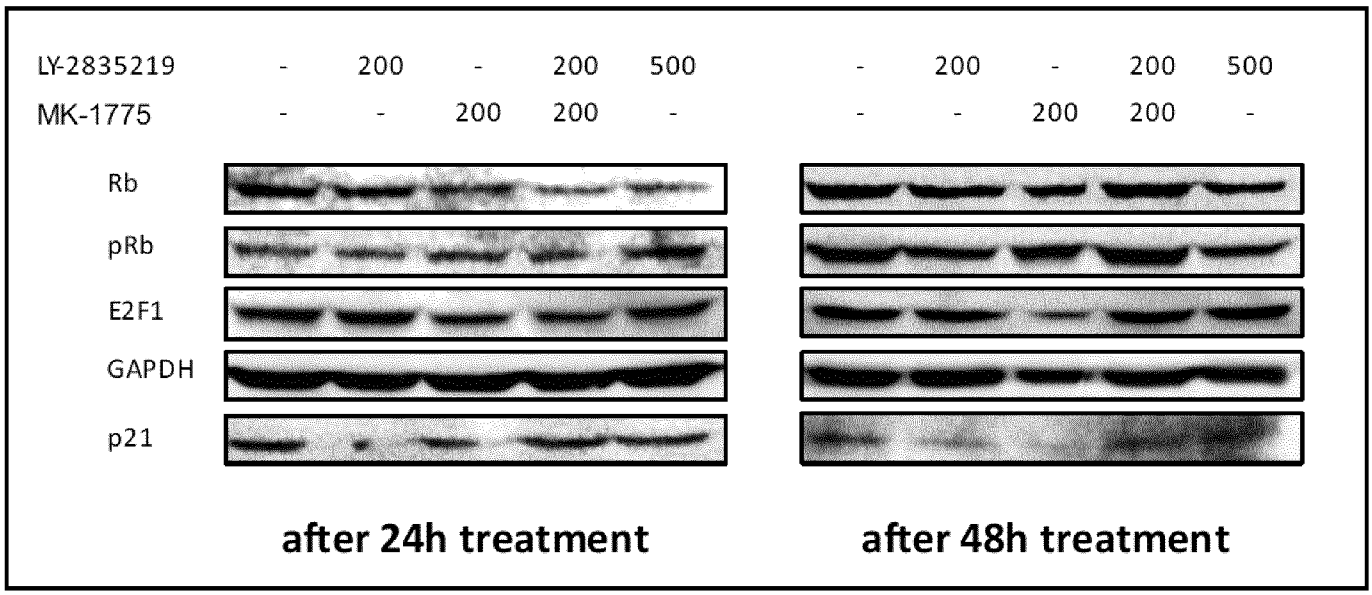

LY-2835219 - 200 - 200 500 - 200 - 200 500
MK-1775 - - 200 200 - - - 200 200 -
Rb
pRb
E2F1
GAPDH
p21
after 24h treatment
after 48h treatment

Fig. 18

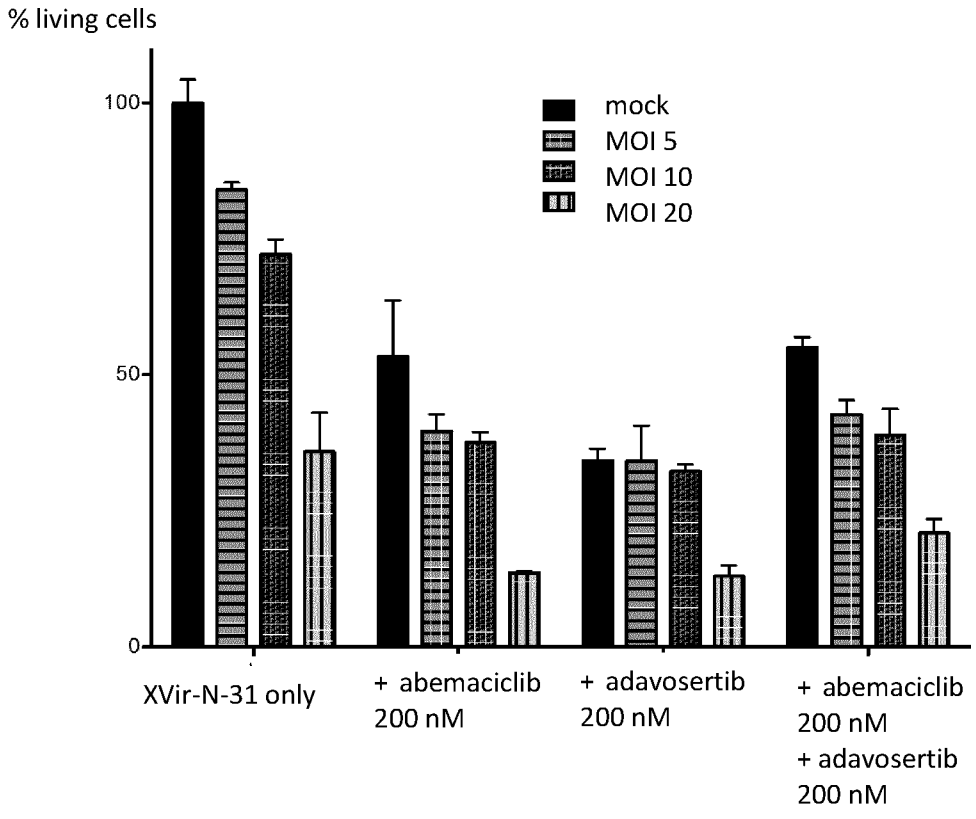

% living cells
100
50
0
mock
MOI 5
MOI 10
MOI 20
XVir-N-31 only
+ abemaciclib 200 nM
+ adavosertib 200 nM
+ abemaciclib 200 nM + adavosertib 200 nM

Fig. 19

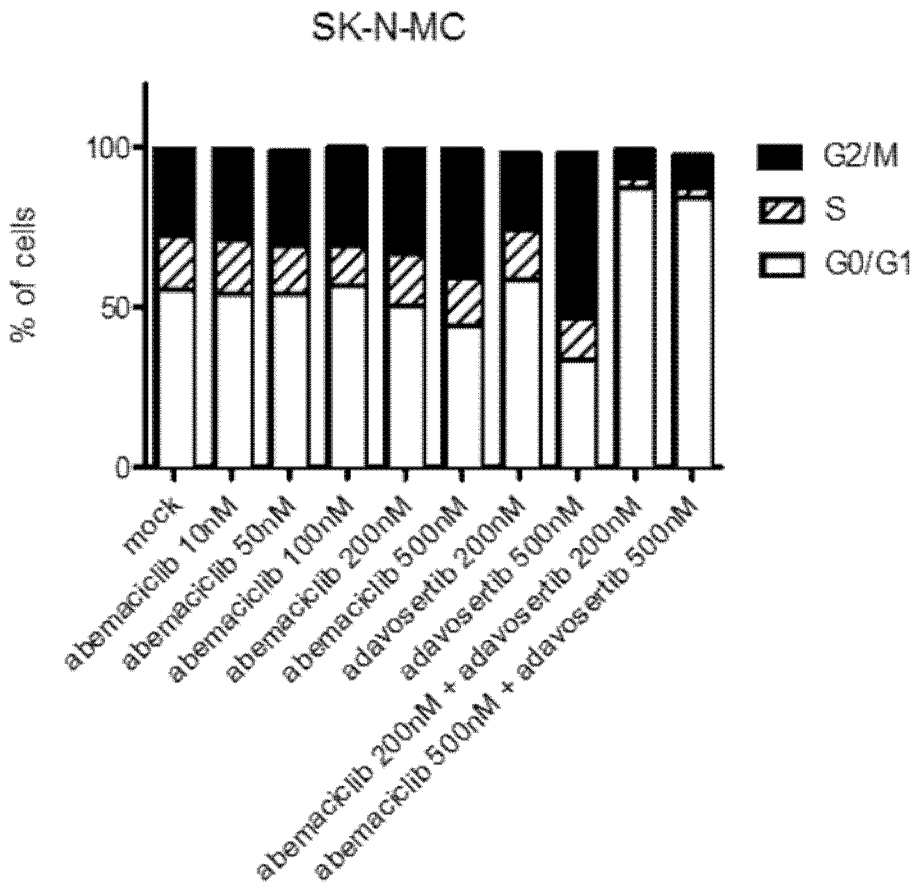

SK-N-MC
% of cells
100
50
0
G2/M
S
G0/G1
mock
abemaciclib 10nM
abemaciclib 50nM
abemaciclib 100nM
abemaciclib 200nM
abemaciclib 500nM
adavosertib 200nM
adavosertib 500nM
abemaciclib 200nM + adavosertib 200nM
abemaciclib 500nM + adavosertib 500nM

Fig. 20

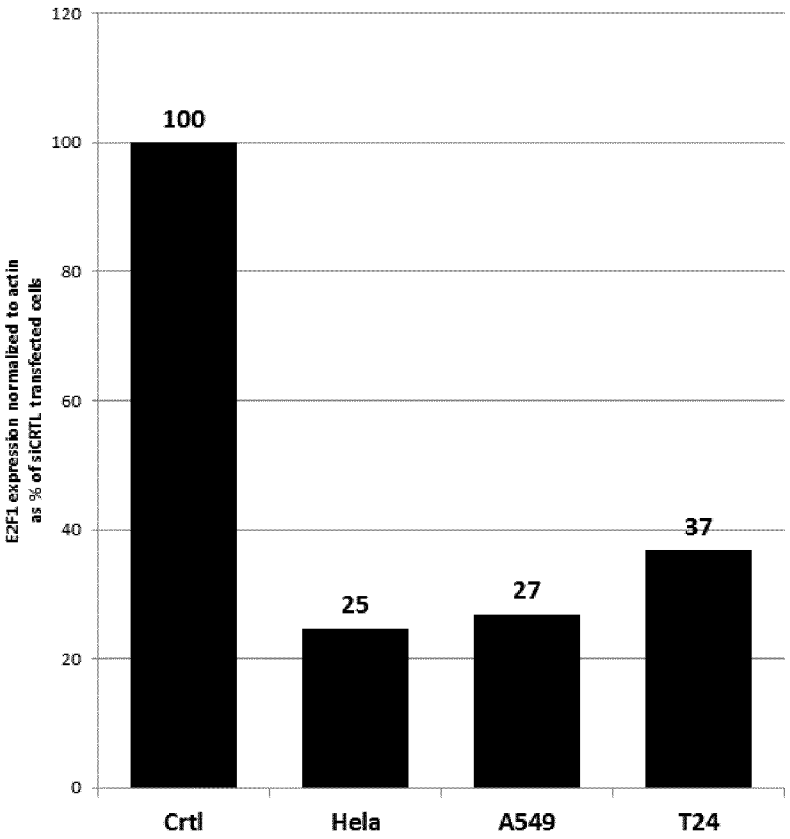

E2F1 expression normalized to actin
as % of siCRTL transfected cells
120
100
80
60
40
20
0
100
25
27
37
Crtl
Hela
A549
T24

Fig. 21

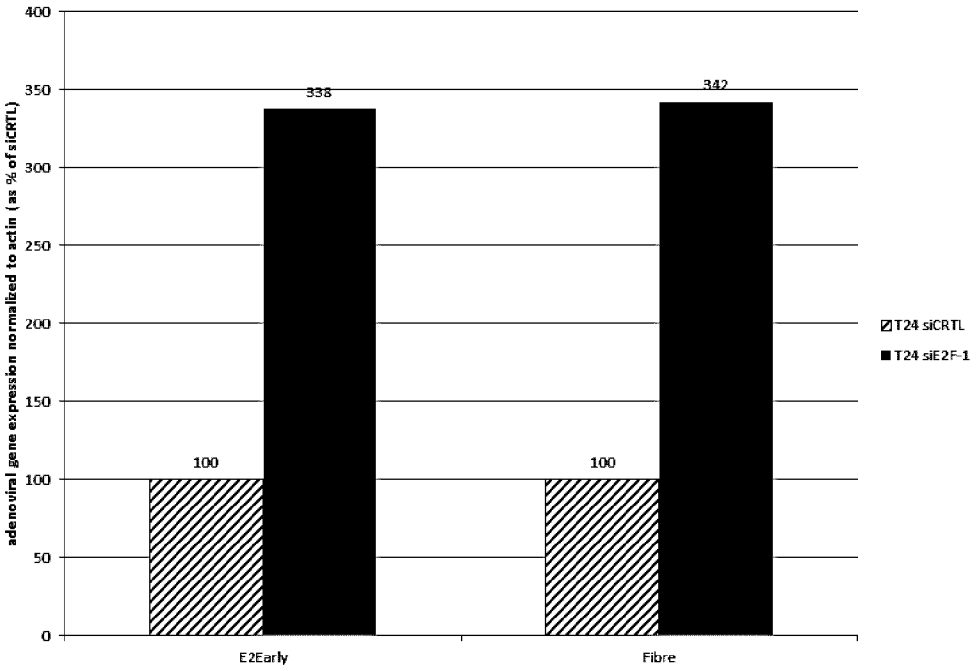


Fig. 22

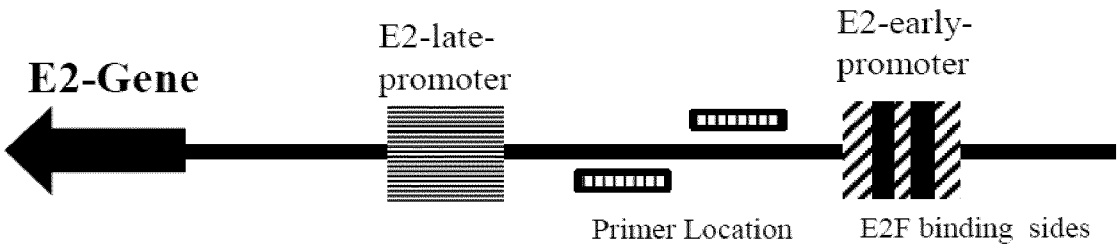


Fig. 23

Wild-type E2-early-promoter

5-AG**TTTTCGCGC**TTAAATTTGAGAAAG**GGCGCGAA**ACTAGTCCTT
3-TC**AAAAGCGCG**AATTTAAACTCTTTC**CCGCGCTT**TGATCAGGAA

5-AG**GAGTCGGAT**TTAAATTTGAGAAAG**TAATAGGT**ACTAGTCCTT
3-TC**CTCAGCCTA**AATTTAAACTCTTTC**ATTATCCA**TGATCAGGAA

Mutated E2-early-promoter

Fig. 24

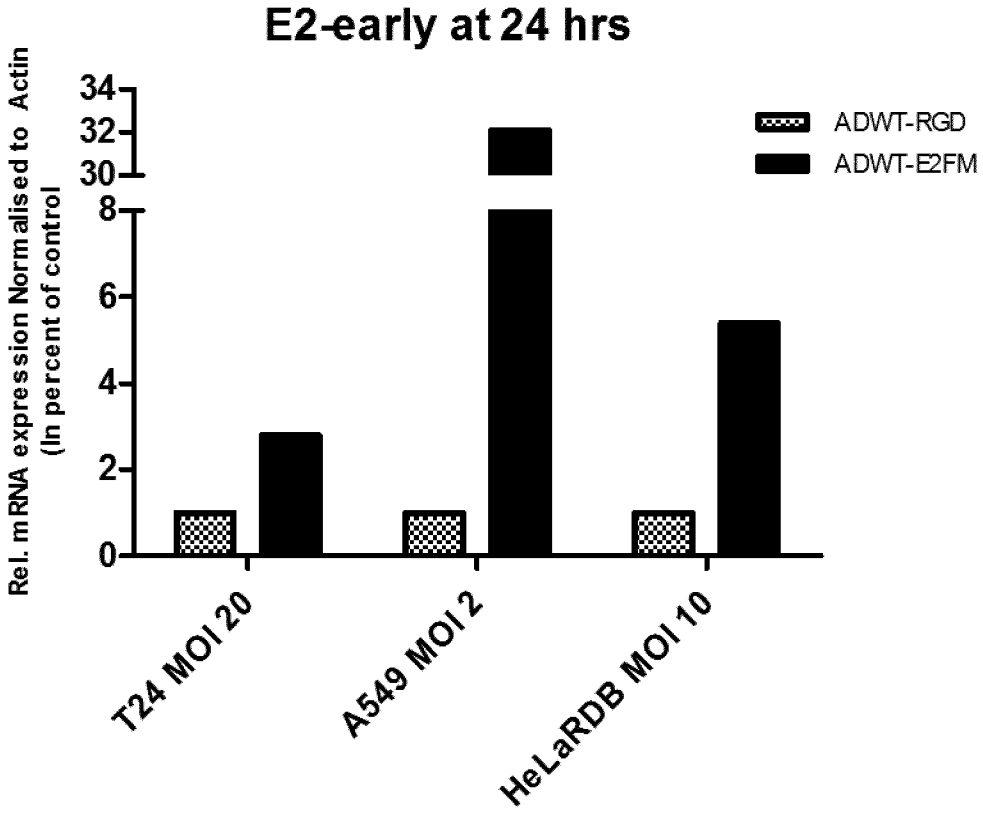
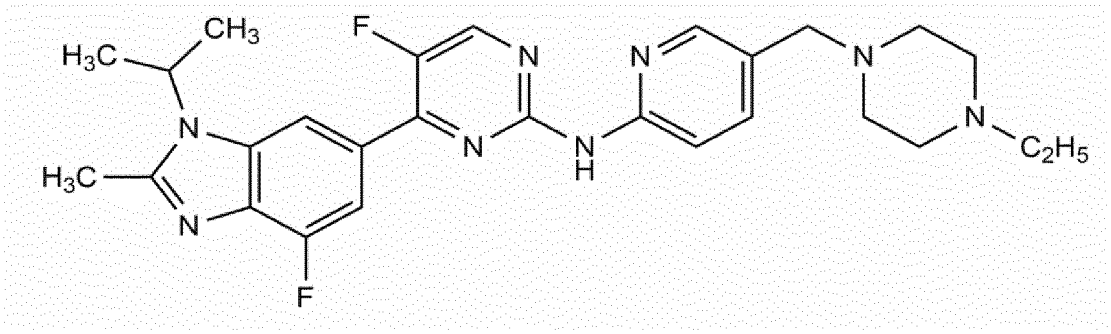
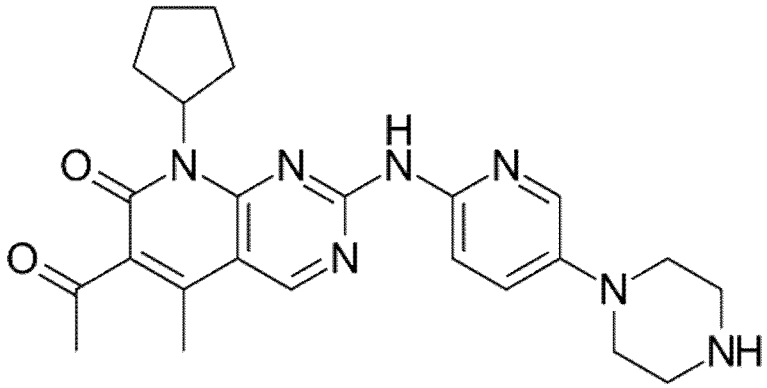

E2-early at 24 hrs
Rel. mRNA expression Normalised to Actin
(In percent of control
34
32
30
8
6
4
2
0
ADWT-RGD
ADWT-E2FM
T24 MOI 20
A549 MOI 2
HeLaRDB MOI 10

Fig. 25

Abemaciclib



Palbociclib



Ribociclib

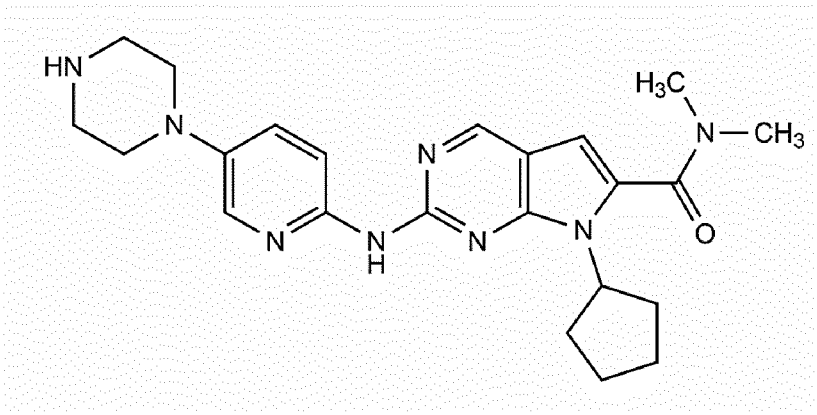


Fig. 25 (continued)

Dinaciclib

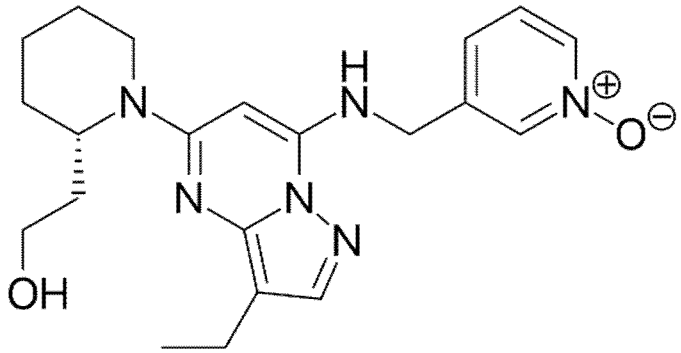
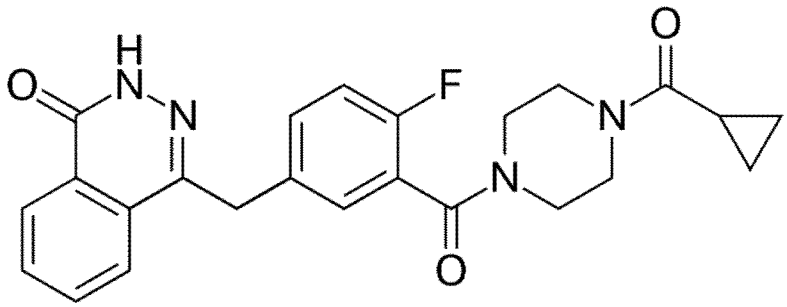
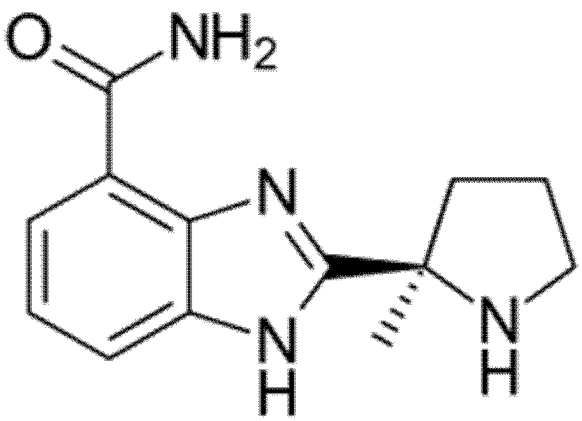


Fig. 26

Olaparib



Veliparib



Rucaparib

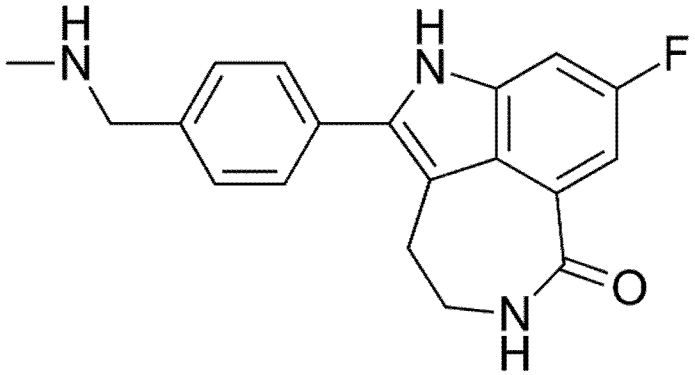


Fig. 26 (continued)

Talazoparib

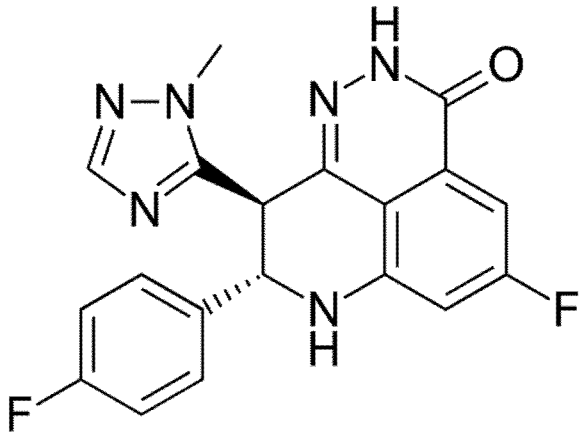
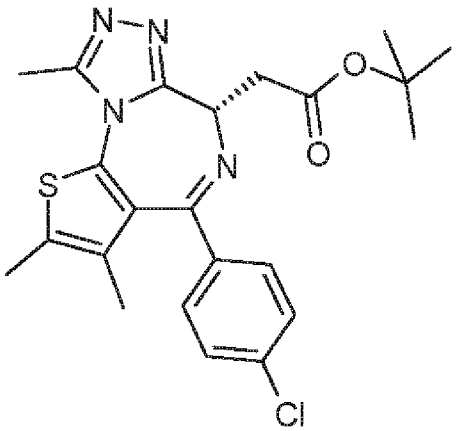


Fig. 27

(Taken from Montserrat Perez-Savia and Manel Esteller: Bromodomain inhibitors and cancer therapy: From structure to applications. EPIGENTCS 2017, 12, 323-339)

JQ1



OTX-015

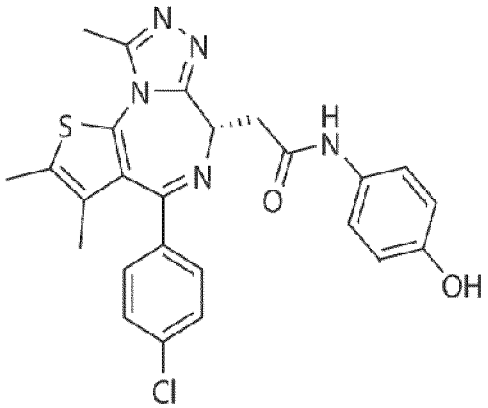
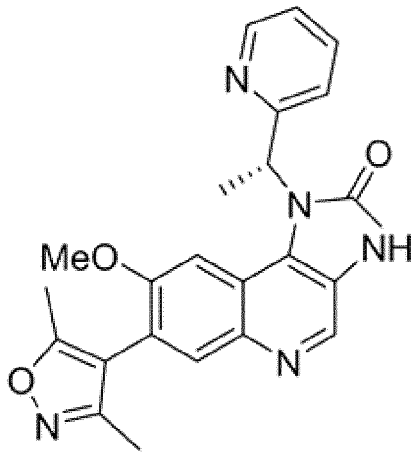
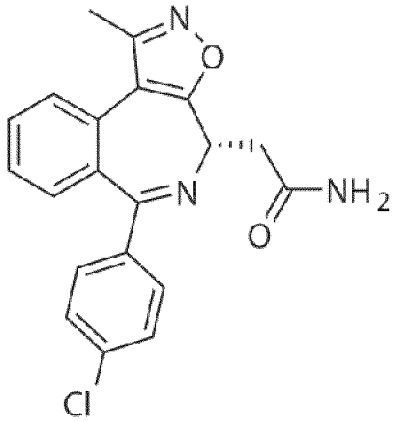


Fig. 27 (continued)

I-BET151



CPI-0610



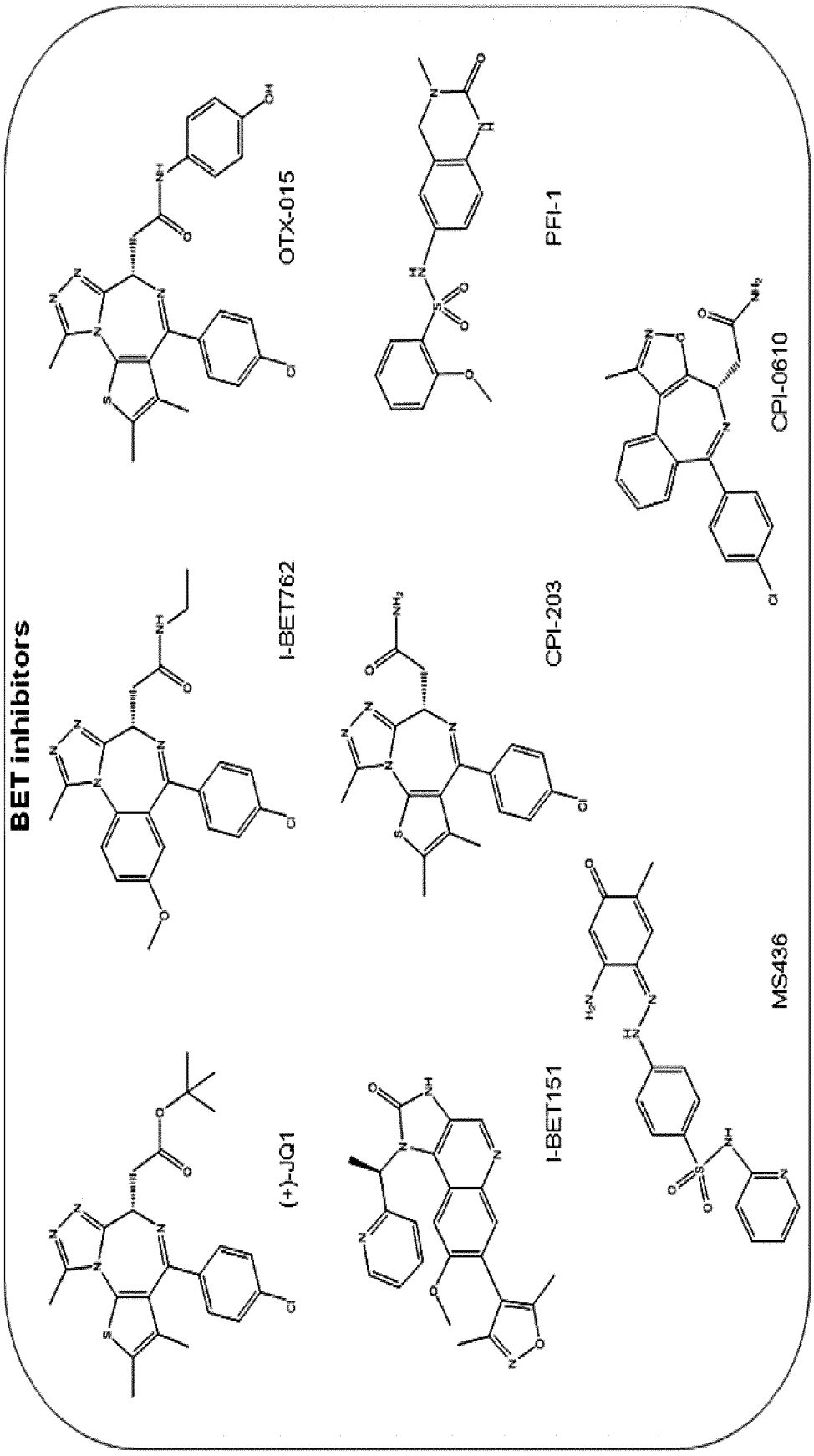


Fig. 27 (continued)

Fig. 28

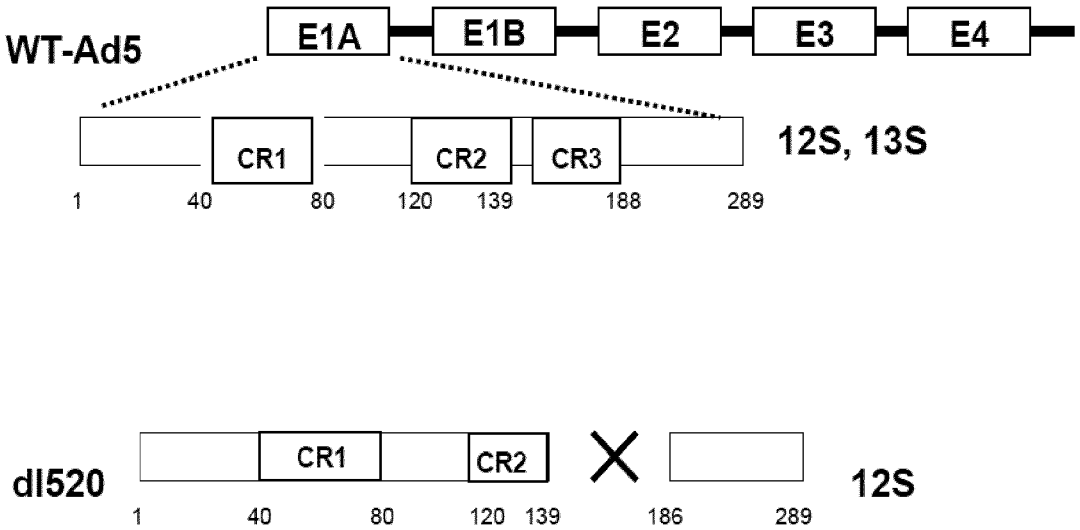

WT-Ad5
E1A
E1B
E2
E3
E4
CR1
CR2
CR3
12S, 13S
1
40
80
120
139
188
289
dl520
CR1
CR2
12S
1
40
80
120
139
186
289

Fig. 29

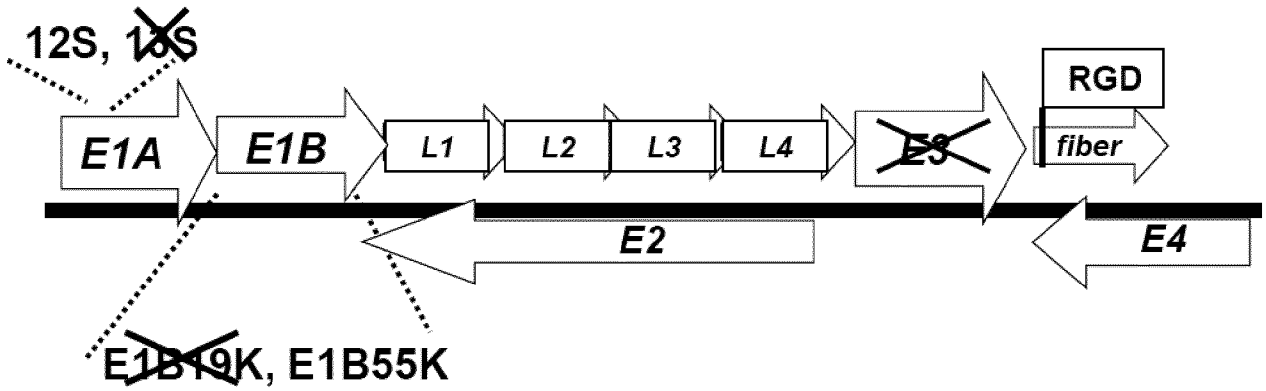

12S, 13S
E1A
E1B
L1
L2
L3
L4
E3
RGD
fiber
E2
E4
E1B19K, E1B55K

Fig. 30

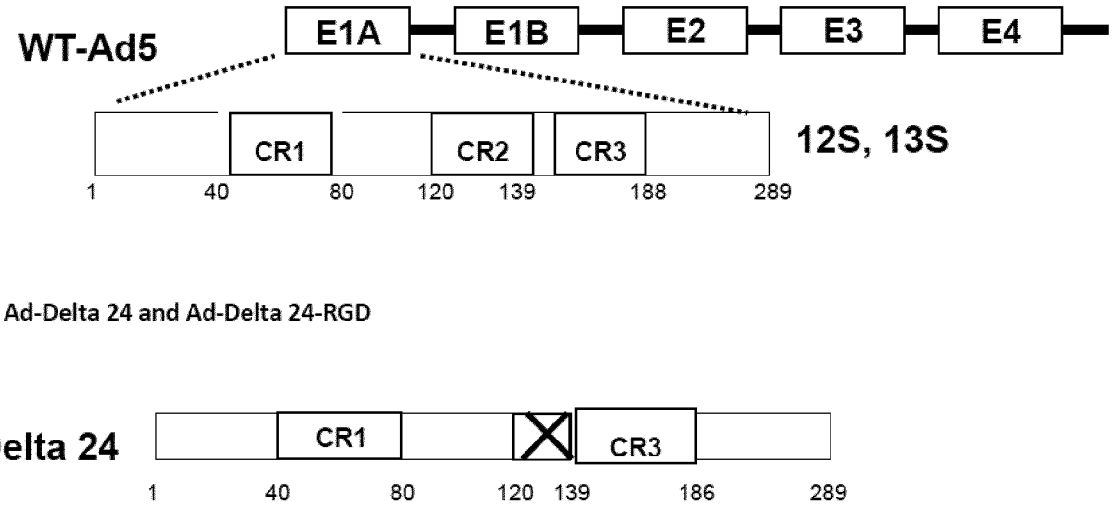


Fig. 31

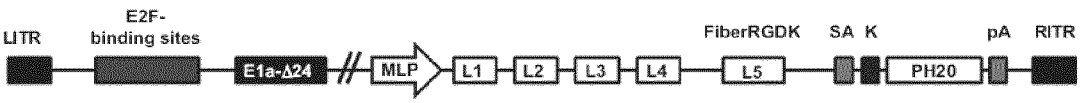


Fig. 32

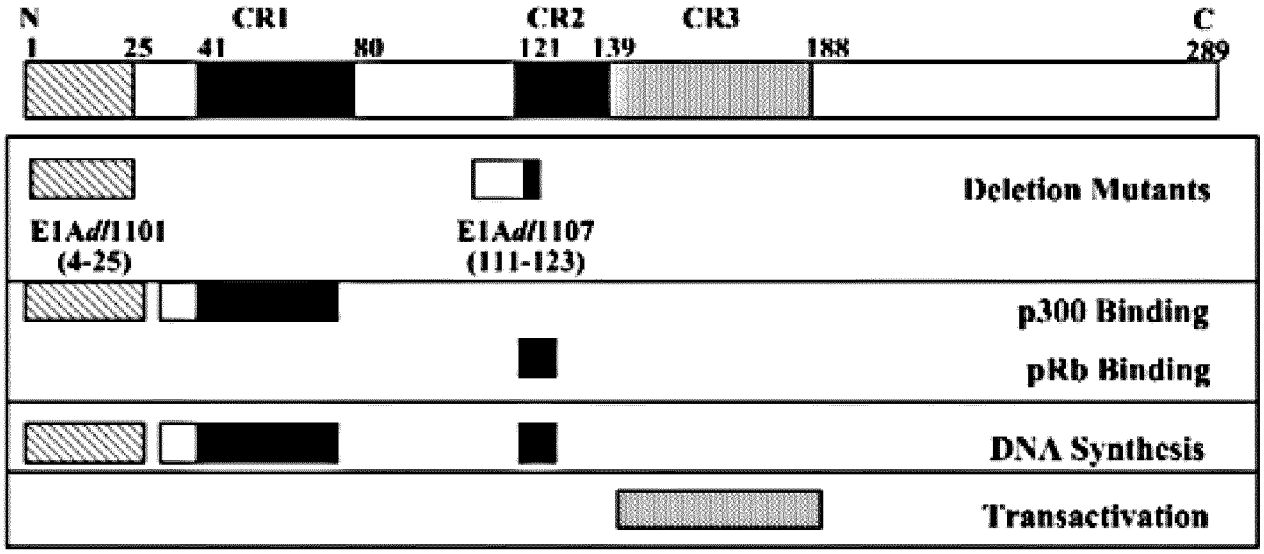


Fig. 33

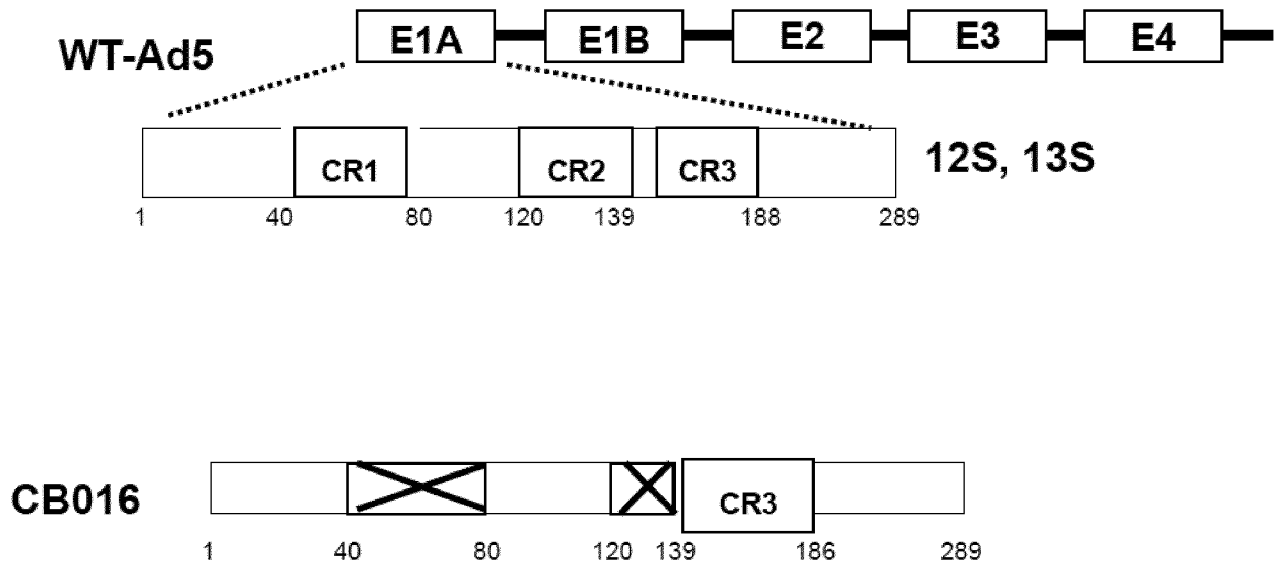

WT-Ad5
E1A
E1B
E2
E3
E4
CR1
CR2
CR3
12S, 13S
1
40
80
120
139
188
289
CB016
CR3
1
40
80
120
139
186
289

Fig. 34

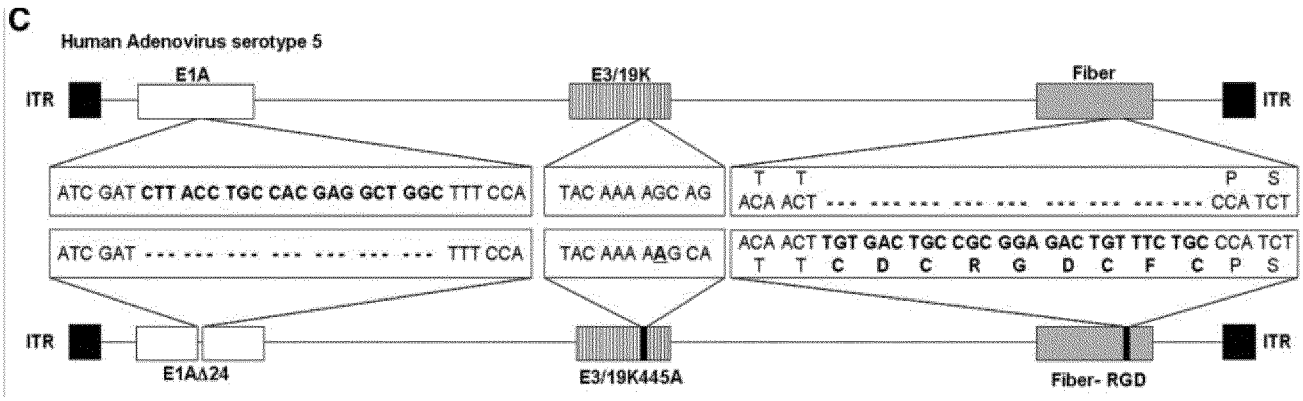

C
Human Adenovirus serotype 5
ITR
E1A
E3/19K
Fiber
ITR
ATC GAT CTT ACC TGC CAC GAG GCT GGC TTT CCA
TAC AAA AGC AG
T T P S
ACA ACT --- --- --- --- --- --- --- --- --- CCA TCT
ATC GAT --- --- --- --- --- --- --- TTT CCA
TAC AAA AAG CA
ACA ACT TGT GAC TGC CGC GGA GAC TGT TTC TGC CCA TCT
T T C D C R G D C F C P S
ITR
E1AΔ24
E3/19K445A
Fiber- RGD
ITR

Fig. 35

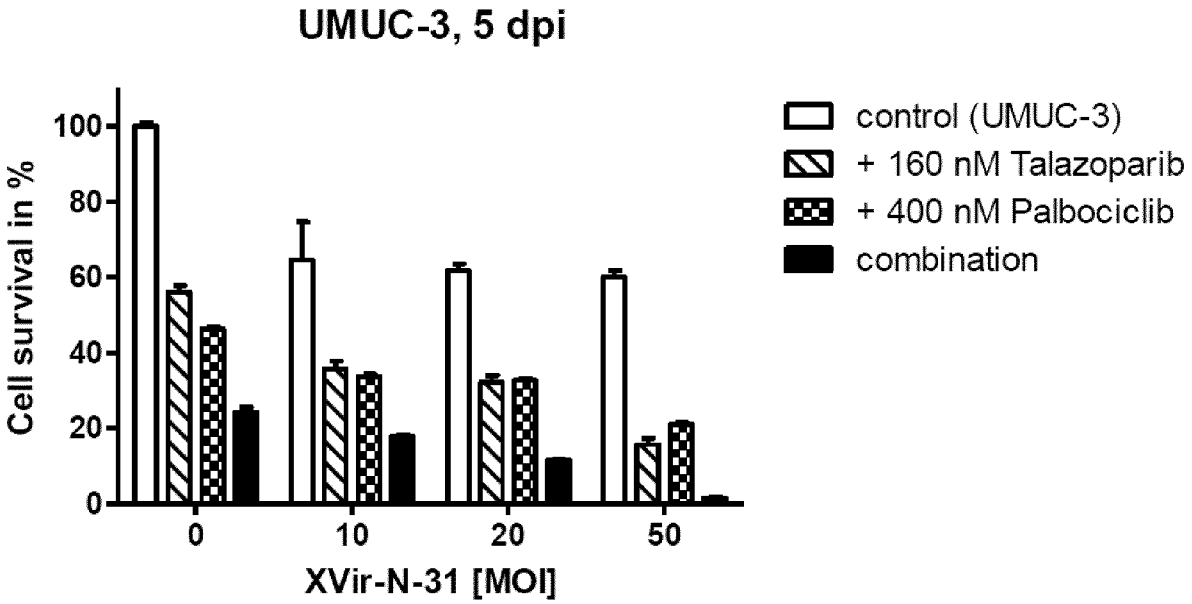

UMUC-3, 5 dpi
Cell survival in %
100
80
60
40
20
0
0
10
20
50
XVir-N-31 [MOI]
control (UMUC-3)
+ 160 nM Talazoparib
+ 400 nM Palbociclib
combination

Fig. 36

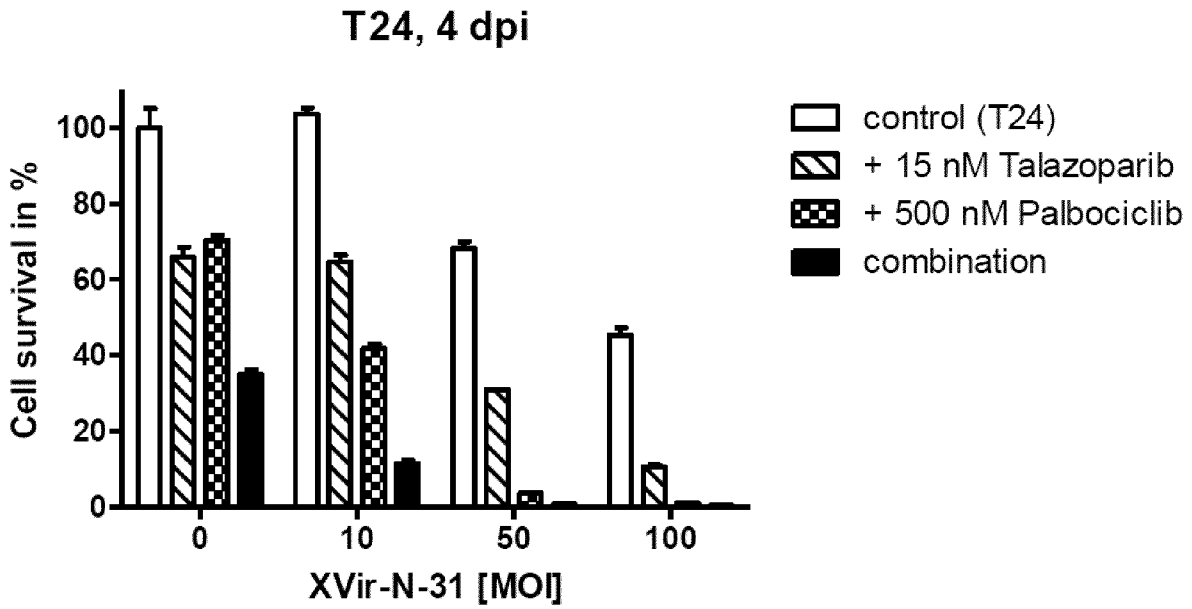

T24, 4 dpi
Cell survival in %
100
80
60
40
20
0
0
10
50
100
XVir-N-31 [MOI]
control (T24)
+ 15 nM Talazoparib
+ 500 nM Palbociclib
combination

Fig. 37

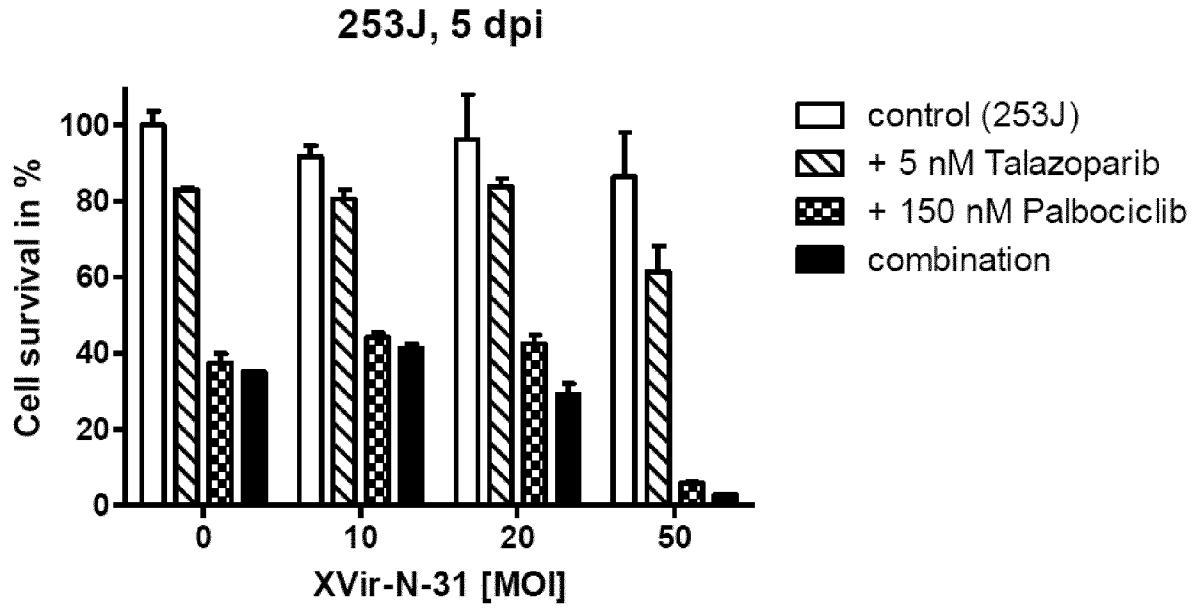

253J, 5 dpi
Cell survival in %
100
80
60
40
20
0
0
10
20
50
XVir-N-31 [MOI]
control (253J)
+ 5 nM Talazoparib
+ 150 nM Palbociclib
combination

Fig. 38

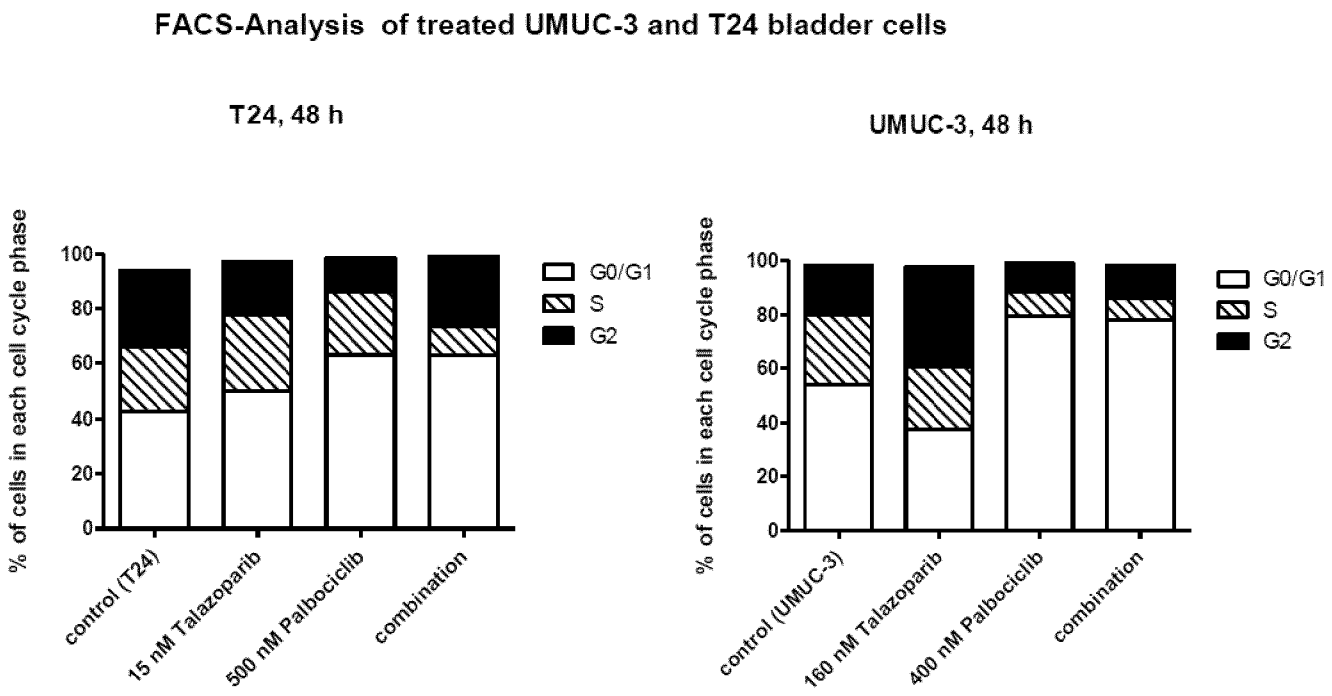

FACS-Analysis of treated UMUC-3 and T24 bladder cells
T24, 48 h
UMUC-3, 48 h
% of cells in each cell cycle phase
100
80
60
40
20
0
G0/G1
S
G2
control (T24)
15 nM Talazoparib
500 nM Palbociclib
combination
control (UMUC-3)
160 nM Talazoparib
400 nM Palbociclib
combination

Fig. 39

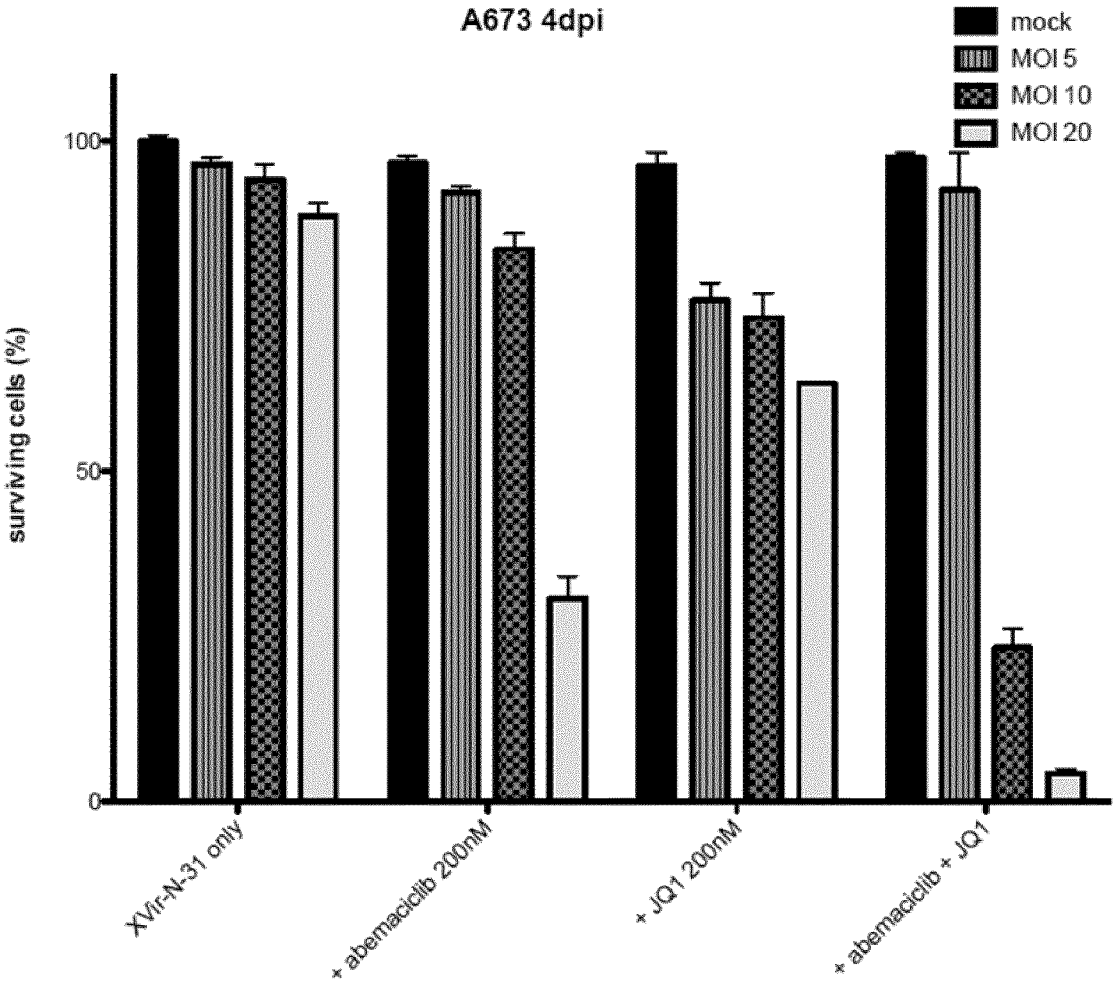

A673 4dpi
mock
MOI 5
MOI 10
MOI 20
surviving cells (%)
100
50
0
XVir-N-31 only
+ abemaciclib 200nM
+ JQ1 200nM
+ abemaciclib + JQ1

Fig. 40

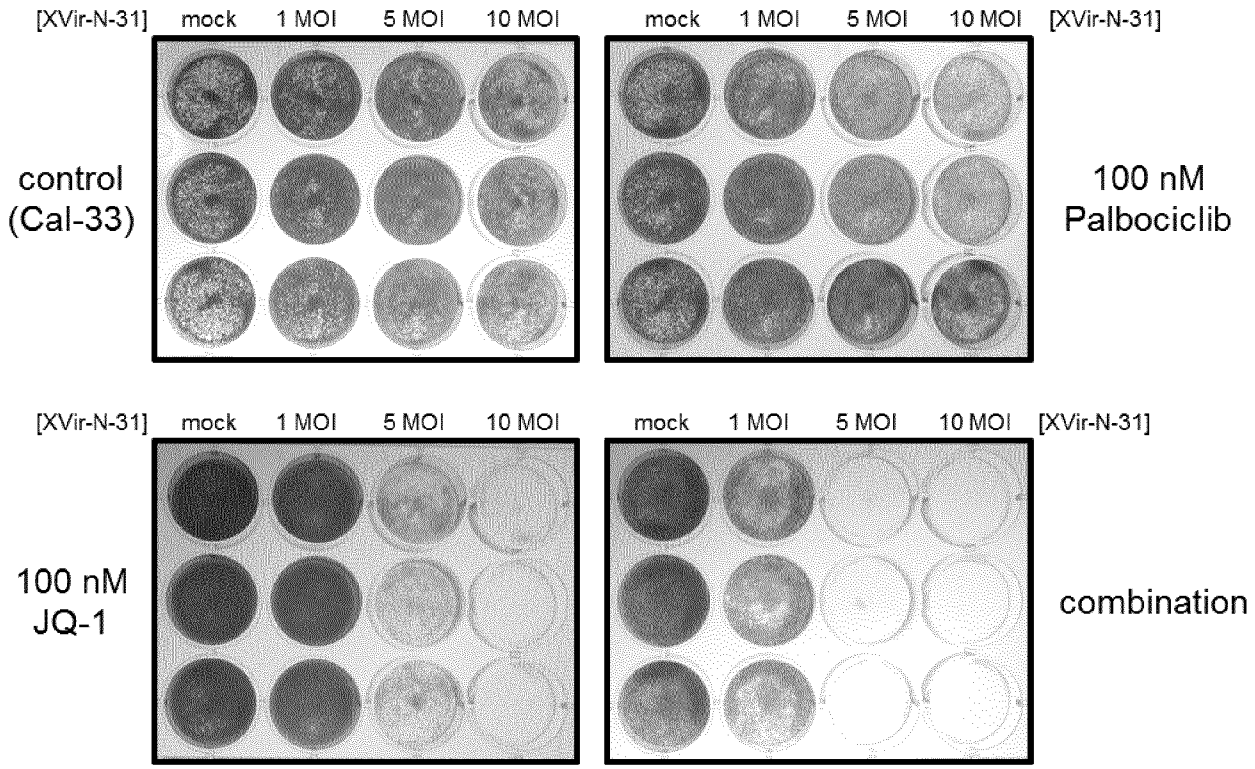

[XVir-N-31]
mock
1 MOI
5 MOI
10 MOI
control (Cal-33)
100 nM Palbociclib
100 nM JQ-1
combination

Fig. 41

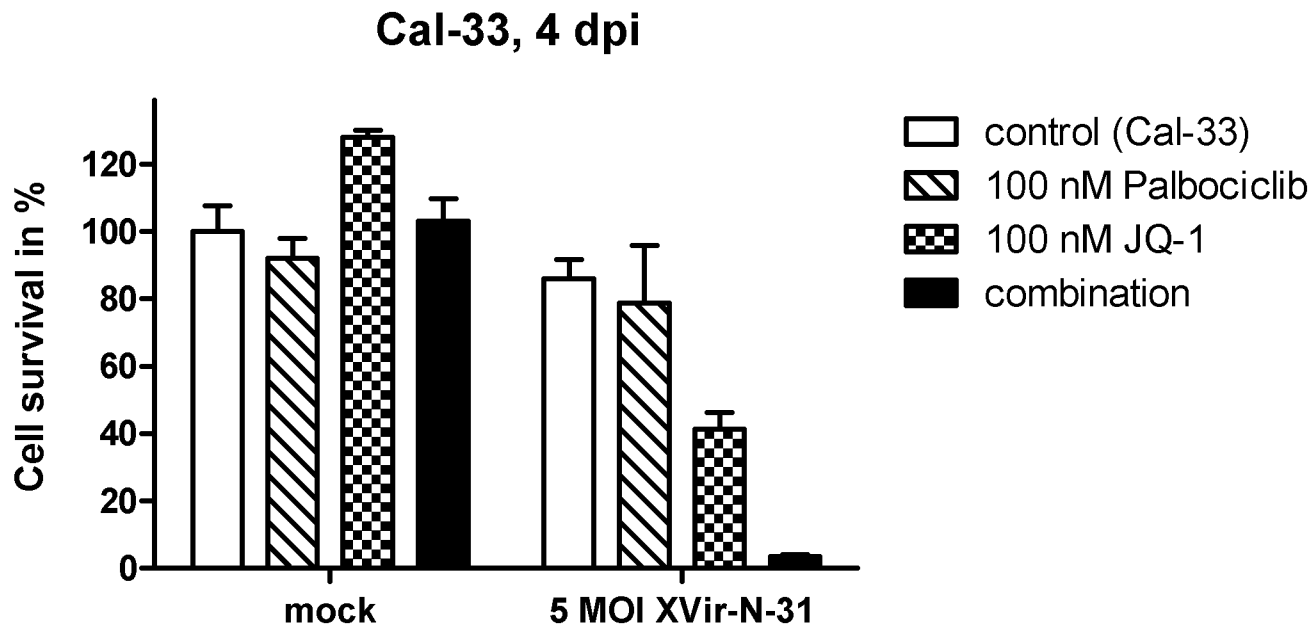

Cal-33, 4 dpi
Cell survival in %
120
100
80
60
40
20
0
mock
5 MOI XVir-N-31
control (Cal-33)
100 nM Palbociclib
100 nM JQ-1
combination

Fig. 42(A)

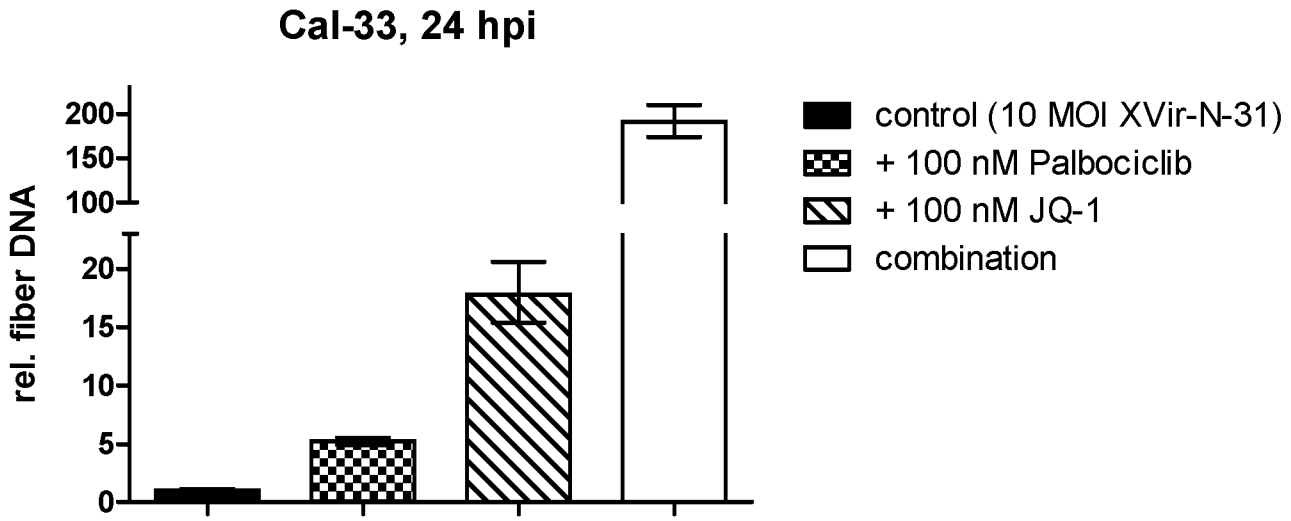


Fig. 42(B)

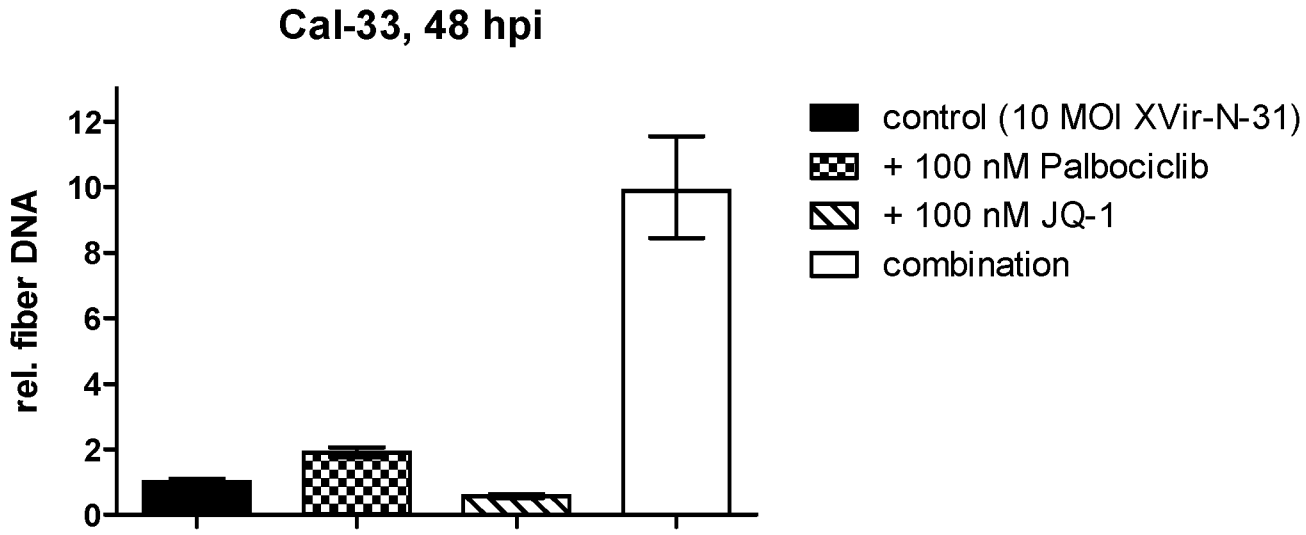
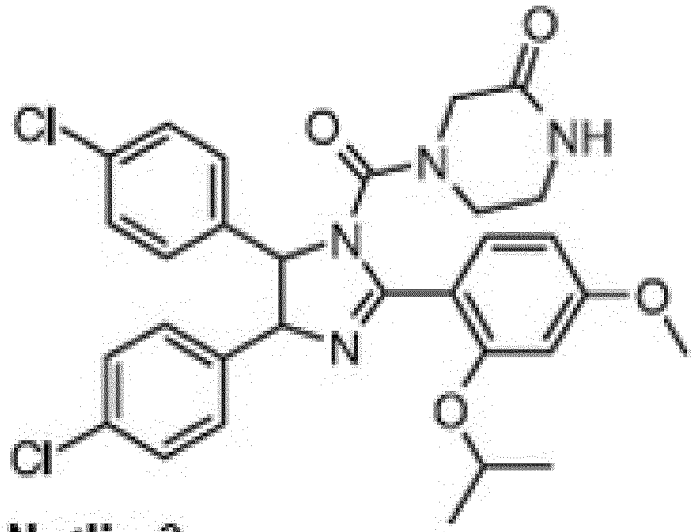


Fig. 43



Nutlin 3

NVP-HDM201 (HDM-201)

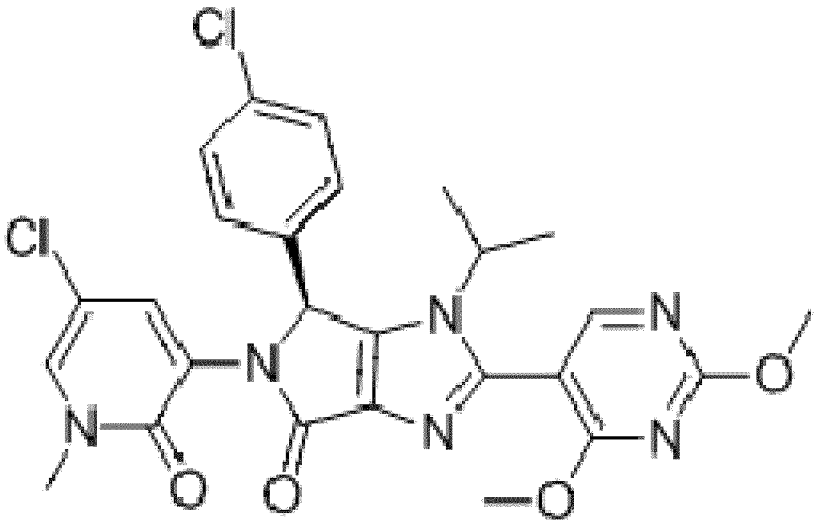
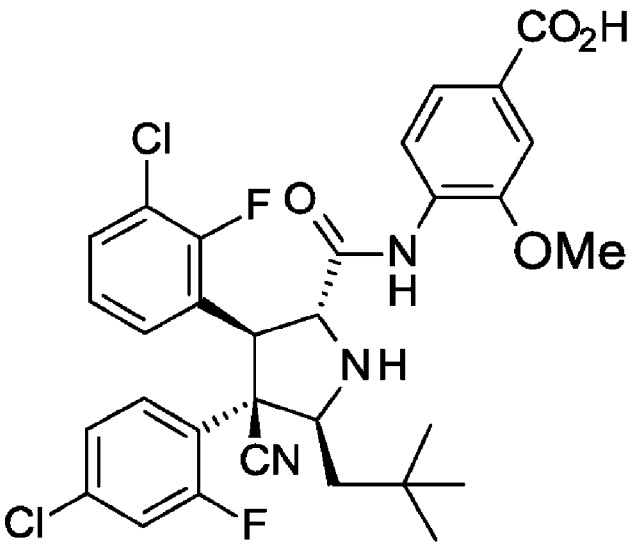


Fig. 43 (continued)

Idasanutlin (RG-7388)



AM-8553

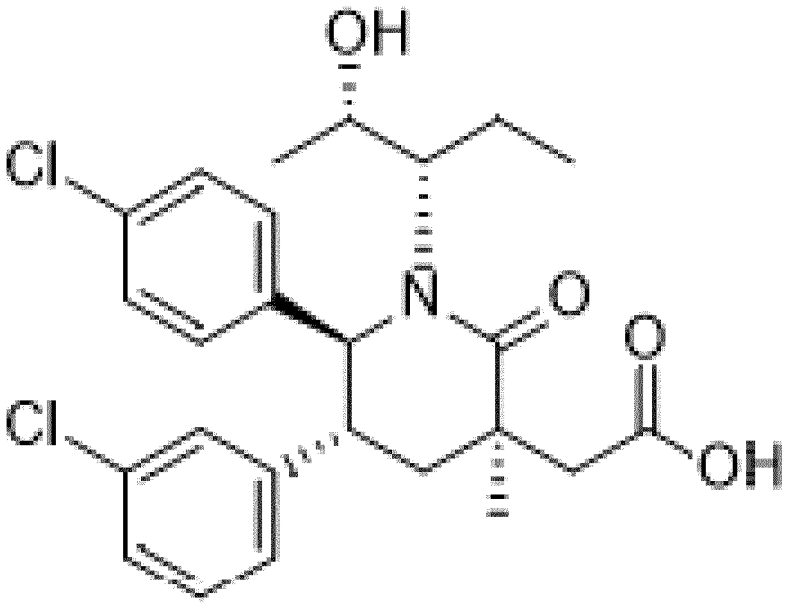
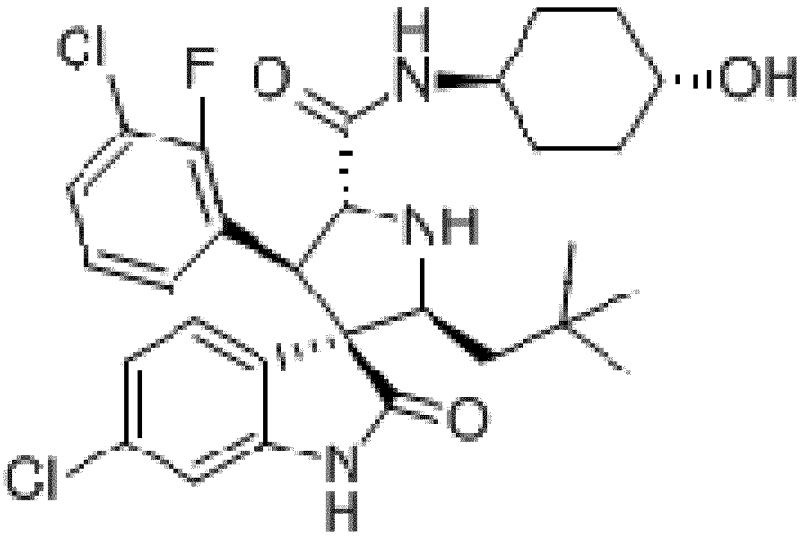


Fig. 43 (continued)

SAR405838 (MI-773)



Nutlin-3a

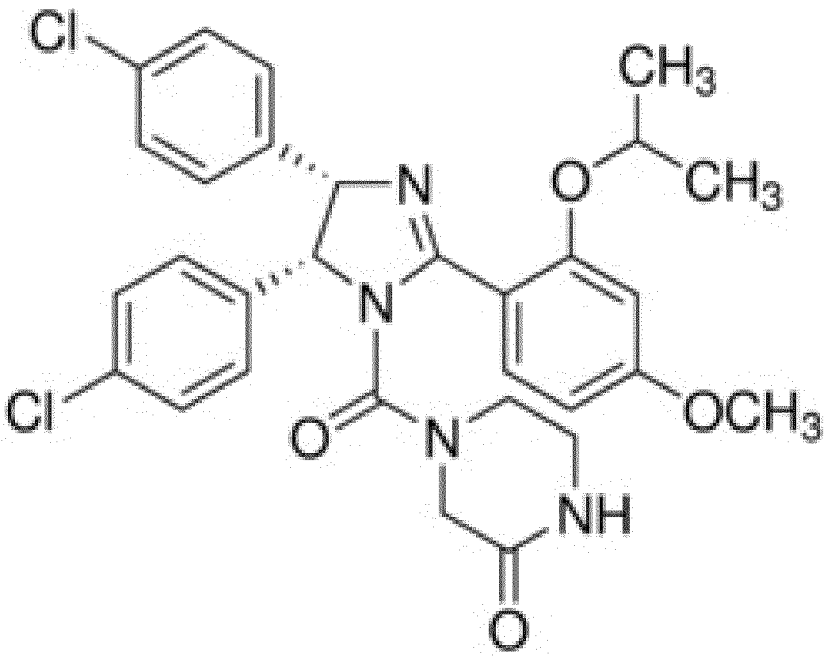


Fig. 43 (continued)

AMG232

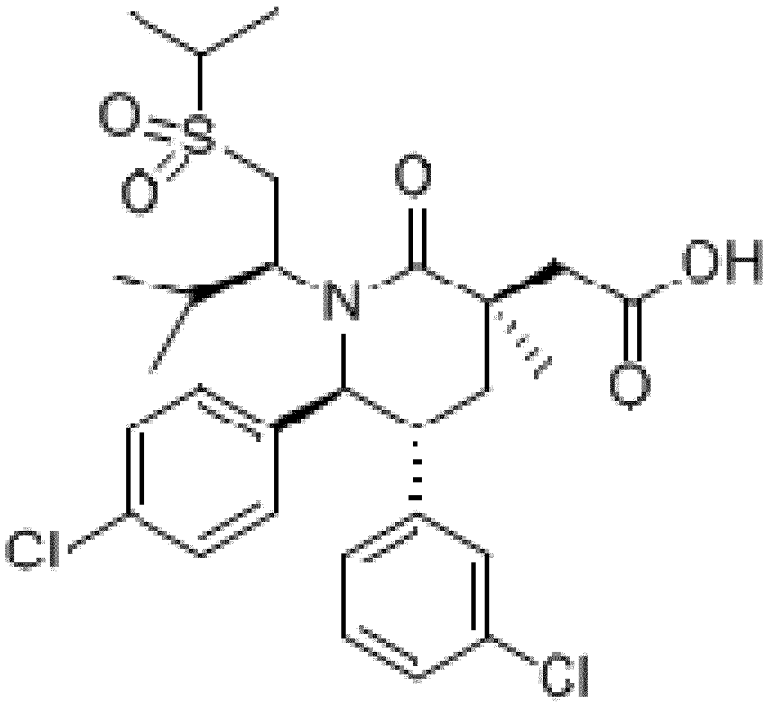


Fig. 44

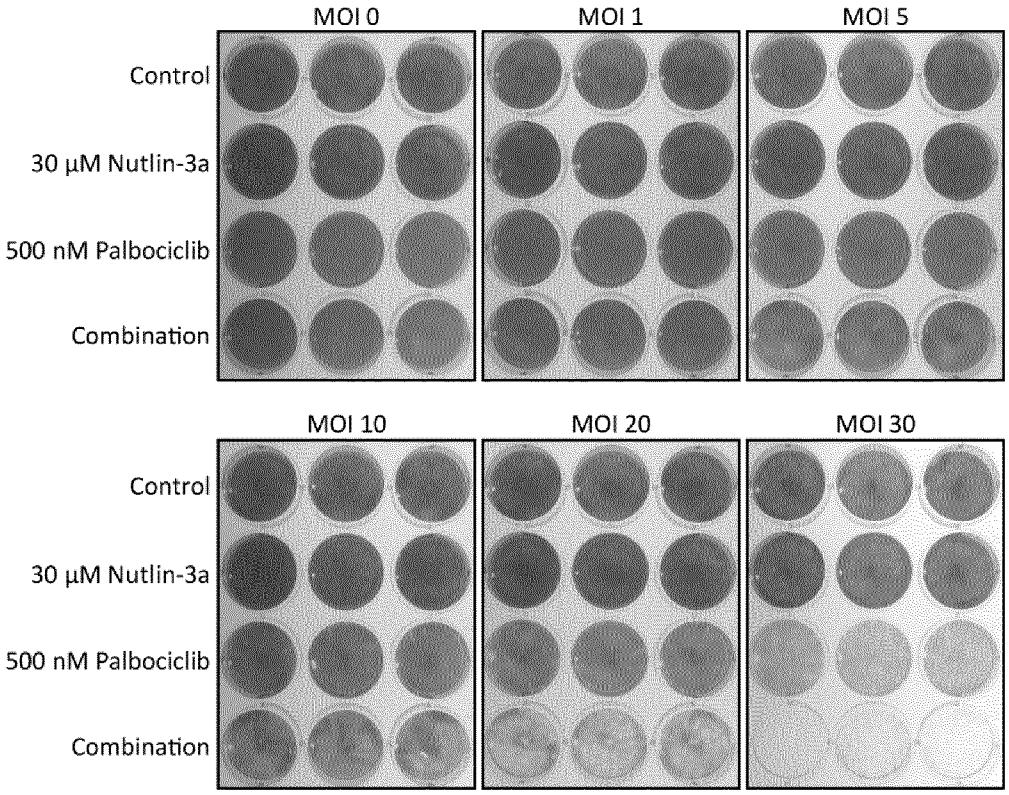

MOI 0
MOI 1
MOI 5
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination
MOI 10
MOI 20
MOI 30
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination

Fig. 45

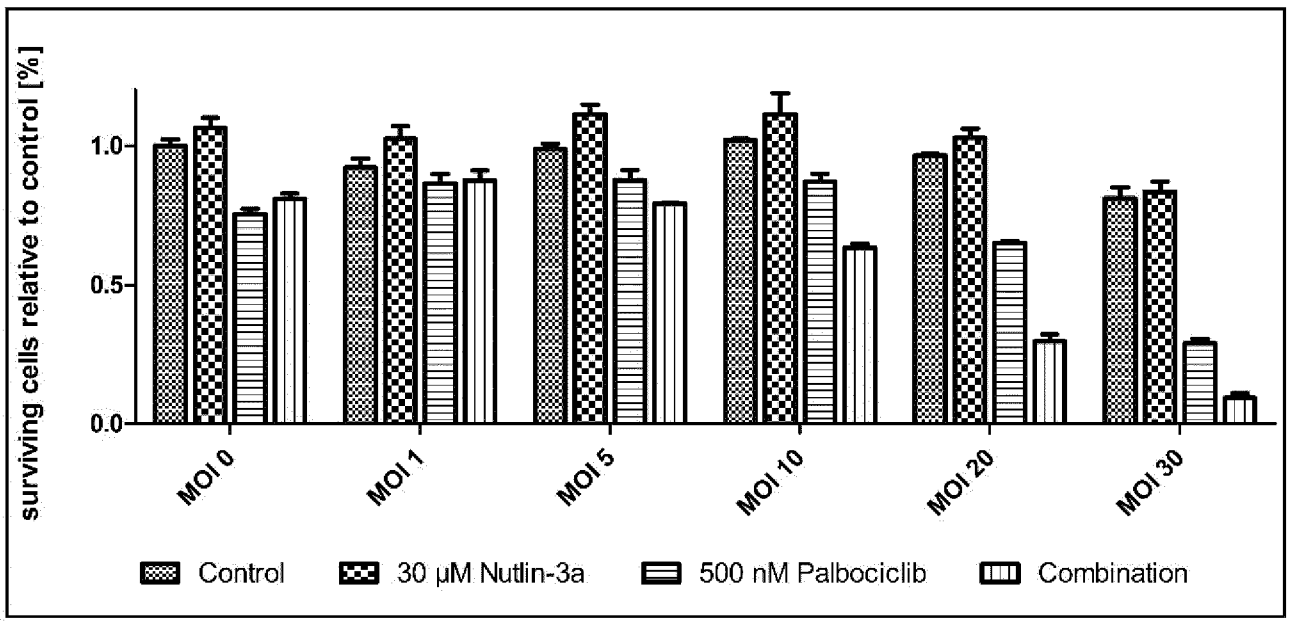

surviving cells relative to control [%]
1.0
0.5
0.0
MOI 0
MOI 1
MOI 5
MOI 10
MOI 20
MOI 30
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination

Fig. 46

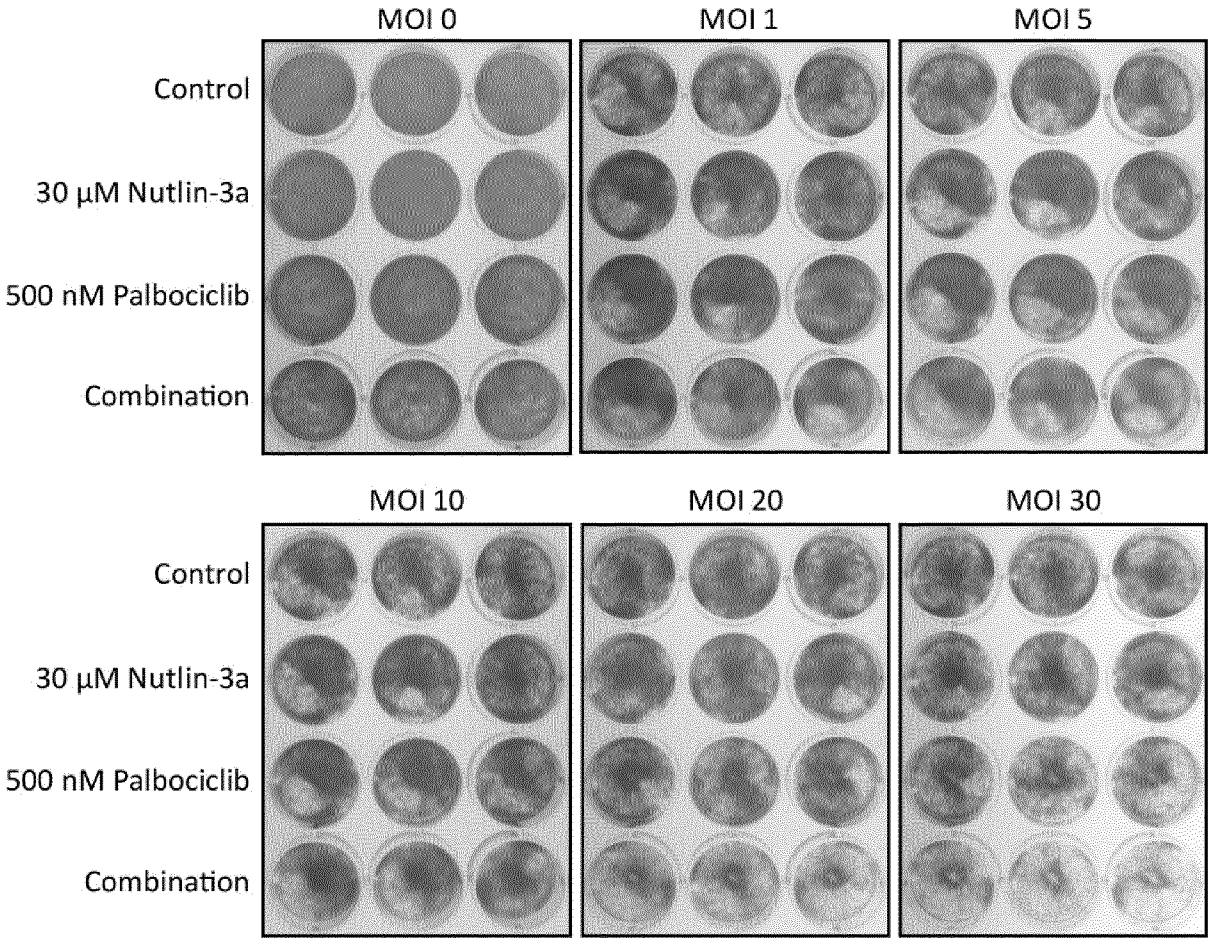

MOI 0
MOI 1
MOI 5
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination
MOI 10
MOI 20
MOI 30
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination

Fig. 47

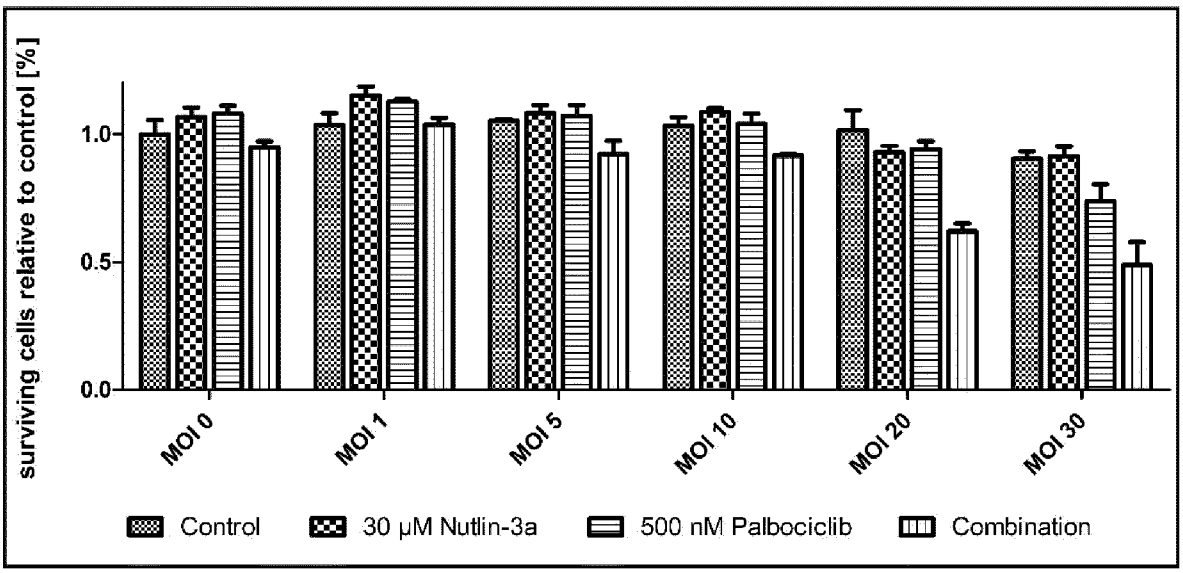

surviving cells relative to control [%]
1.0
0.5
0.0
MOI 0
MOI 1
MOI 5
MOI 10
MOI 20
MOI 30
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination

Fig. 48

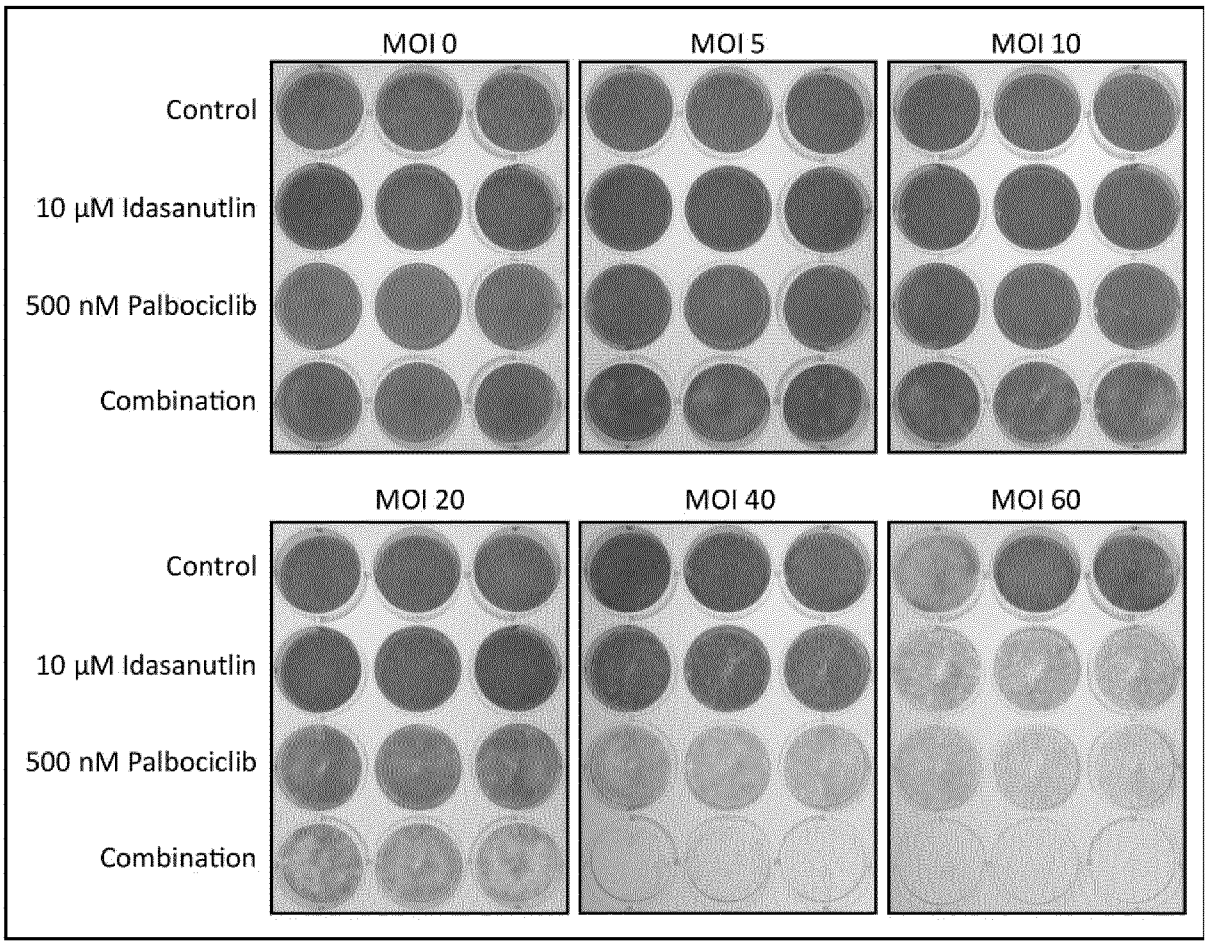

MOI 0
MOI 5
MOI 10
Control
10 µM Idasanutlin
500 nM Palbociclib
Combination
MOI 20
MOI 40
MOI 60
Control
10 µM Idasanutlin
500 nM Palbociclib
Combination

Fig. 49

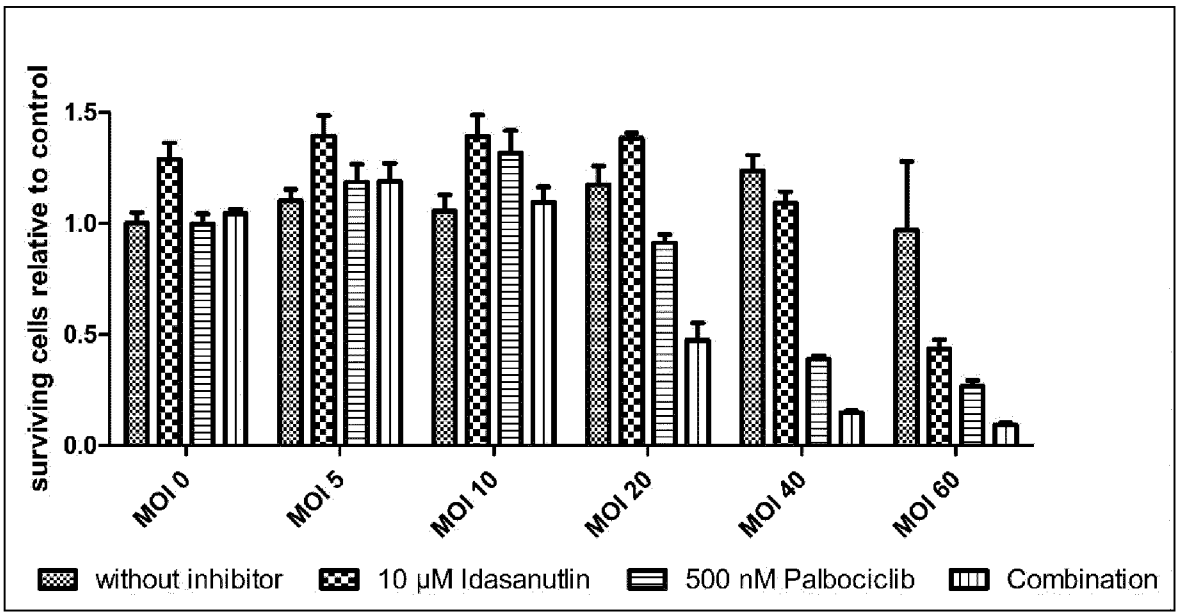

surviving cells relative to control
1.5
1.0
0.5
0.0
MOI 0
MOI 5
MOI 10
MOI 20
MOI 40
MOI 60
without inhibitor
10 µM Idasanutlin
500 nM Palbociclib
Combination

Fig. 50

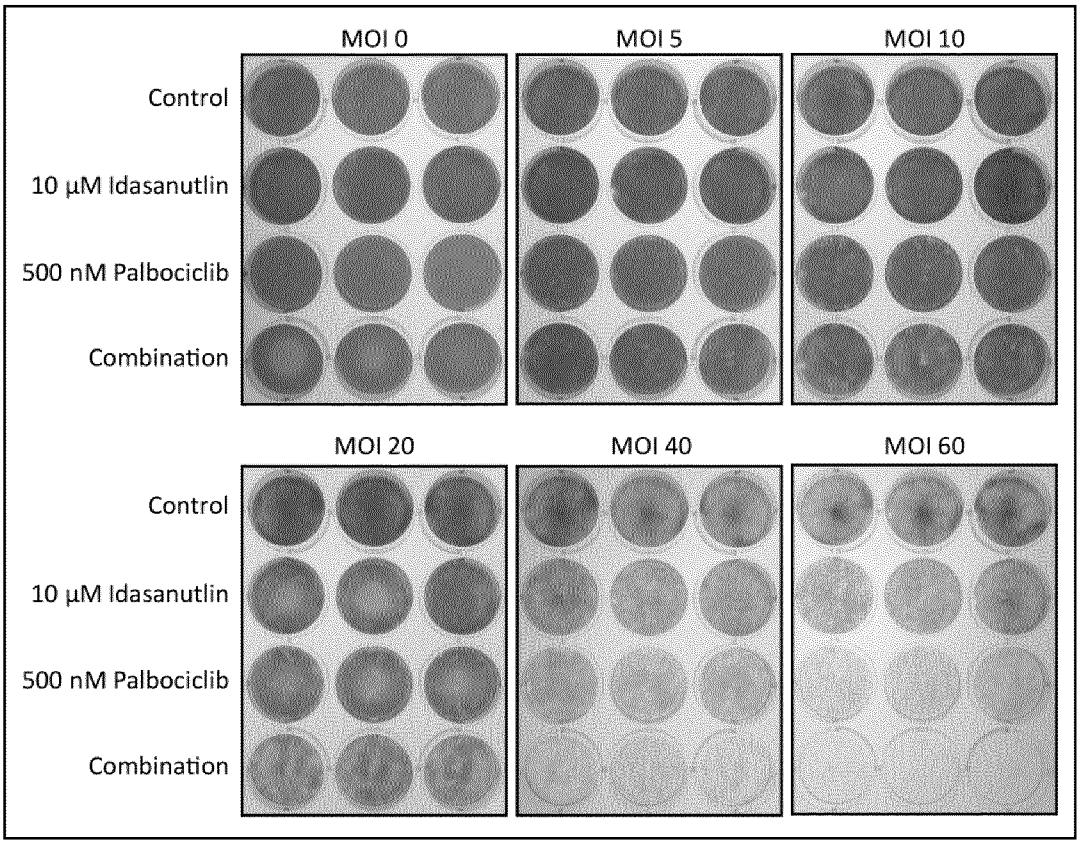

MOI 0
MOI 5
MOI 10
Control
10 µM Idasanutlin
500 nM Palbociclib
Combination
MOI 20
MOI 40
MOI 60
Control
10 µM Idasanutlin
500 nM Palbociclib
Combination

Fig. 51

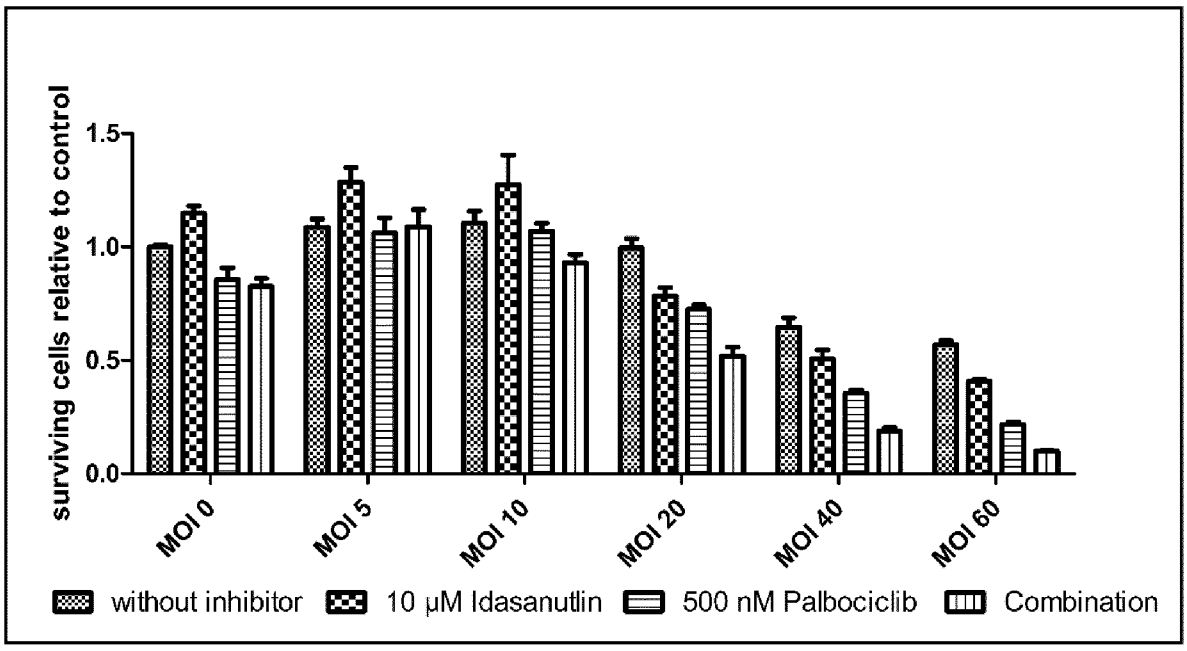

surviving cells relative to control
1.5
1.0
0.5
0.0
MOI 0
MOI 5
MOI 10
MOI 20
MOI 40
MOI 60
without inhibitor
10 µM Idasanutlin
500 nM Palbociclib
Combination

Fig. 52

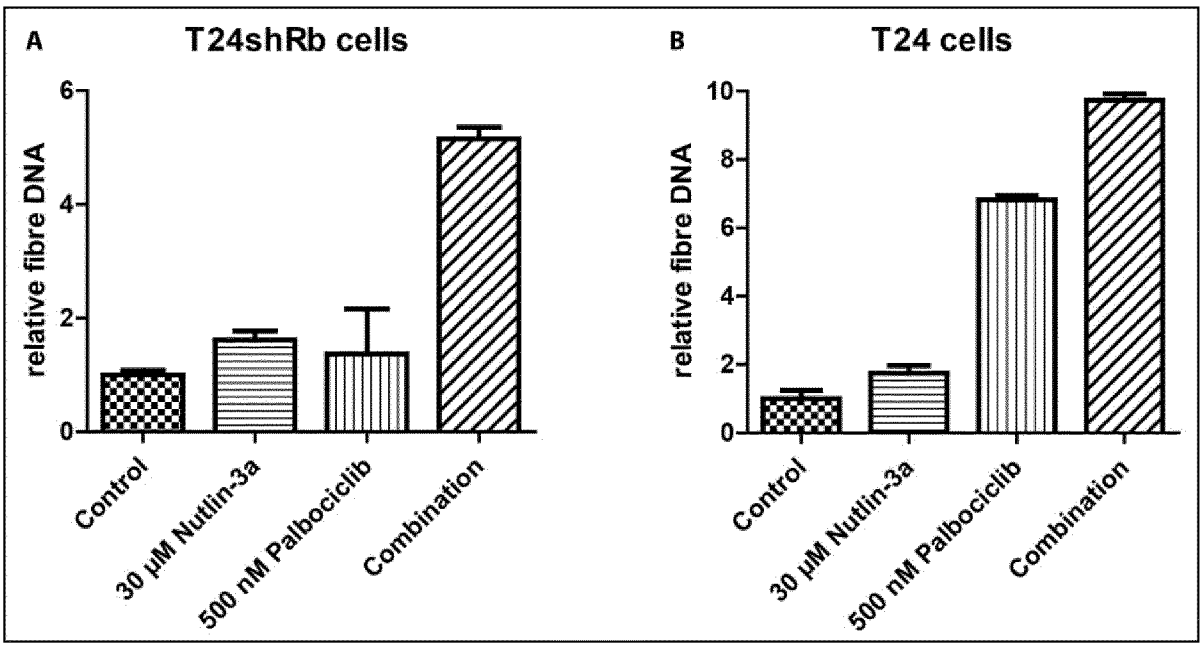

A
T24shRb cells
relative fibre DNA
0
2
4
6
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination
B
T24 cells
relative fibre DNA
0
2
4
6
8
10
Control
30 µM Nutlin-3a
500 nM Palbociclib
Combination

Fig. 53

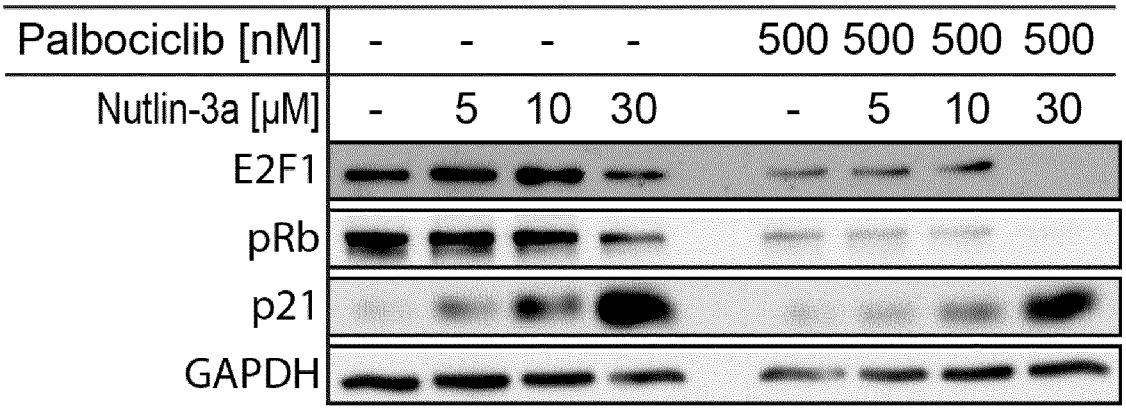

Palbociclib [nM]
- - - - 500 500 500 500
Nutlin-3a [µM]
- 5 10 30 - 5 10 30
E2F1
pRb
p21
GAPDH

Fig. 54

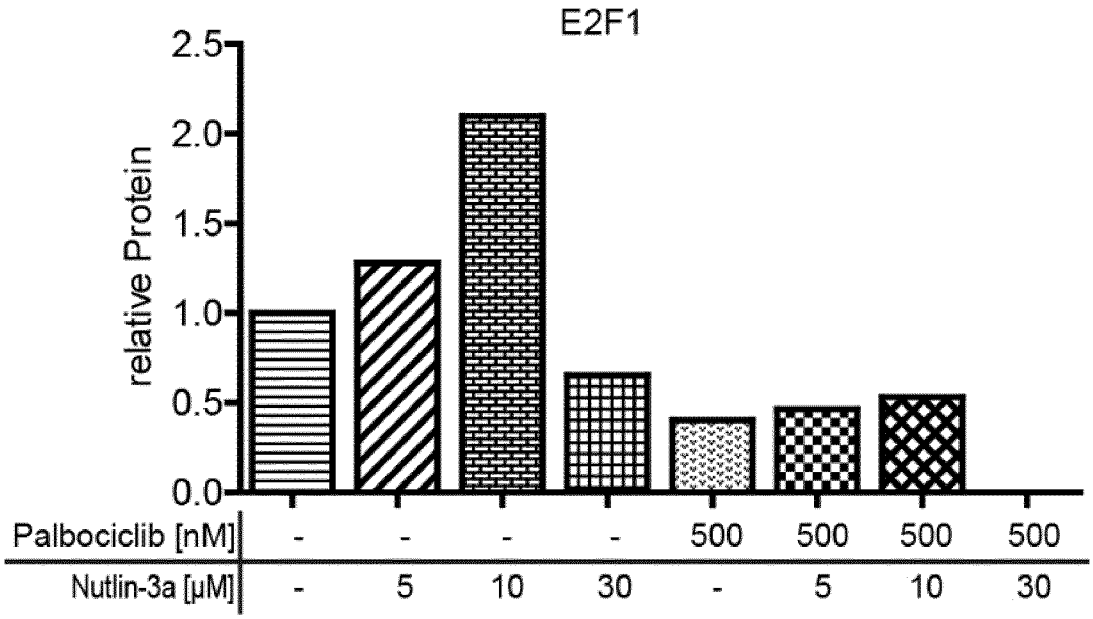

E2F1
relative Protein
2.5
2.0
1.5
1.0
0.5
0.0
Palbociclib [nM] - - - - 500 500 500 500
Nutlin-3a [µM] - 5 10 30 - 5 10 30

Fig. 55

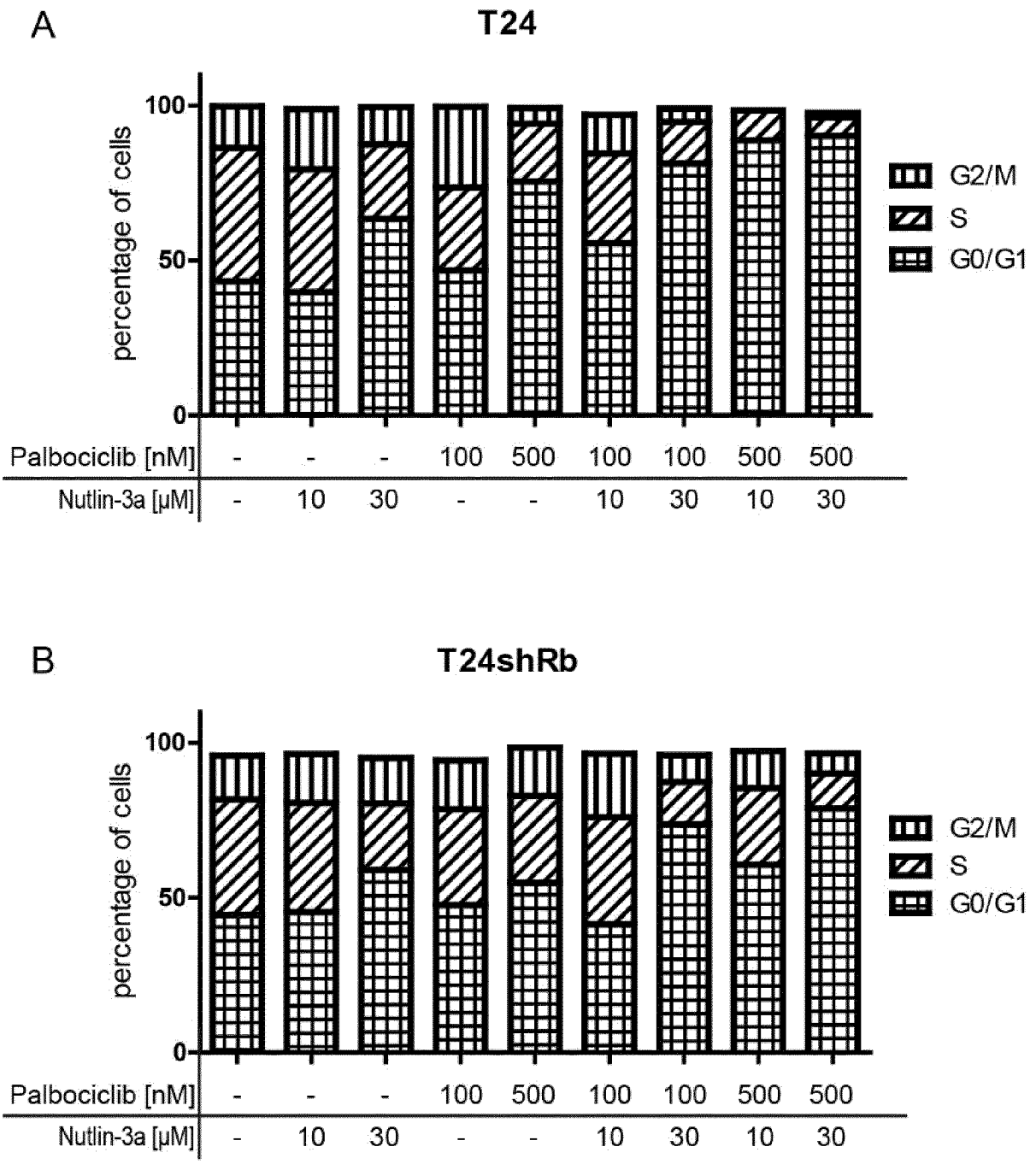

A
T24
percentage of cells
100
50
0
G2/M
S
G0/G1
Palbociclib [nM] - - - 100 500 100 100 500 500
Nutlin-3a [µM] - 10 30 - - 10 30 10 30
B
T24shRb
percentage of cells
100
50
0
G2/M
S
G0/G1
Palbociclib [nM] - - - 100 500 100 100 500 500
Nutlin-3a [µM] - 10 30 - - 10 30 10 30

Fig. 55 (continued)

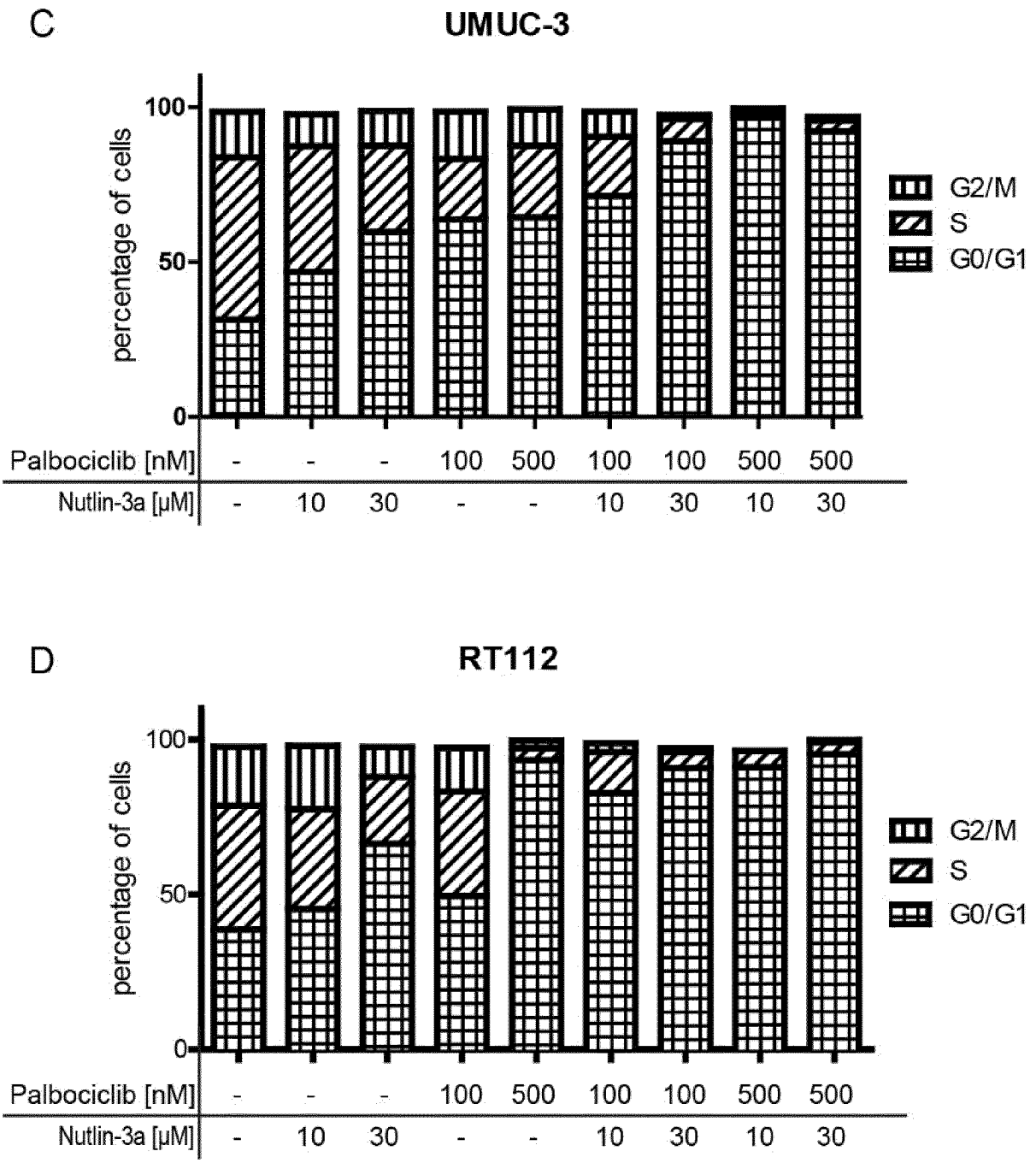


Fig. 56

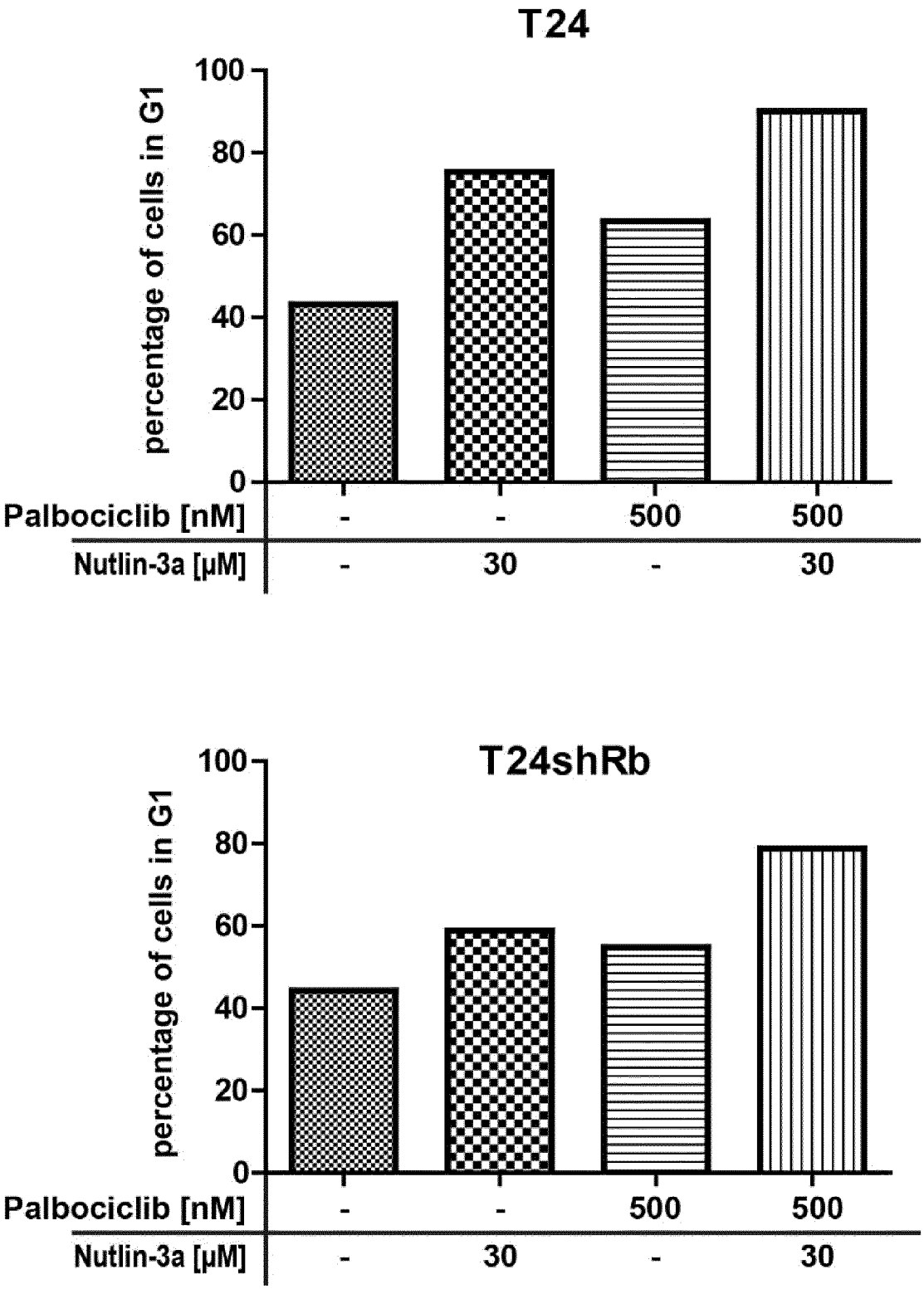
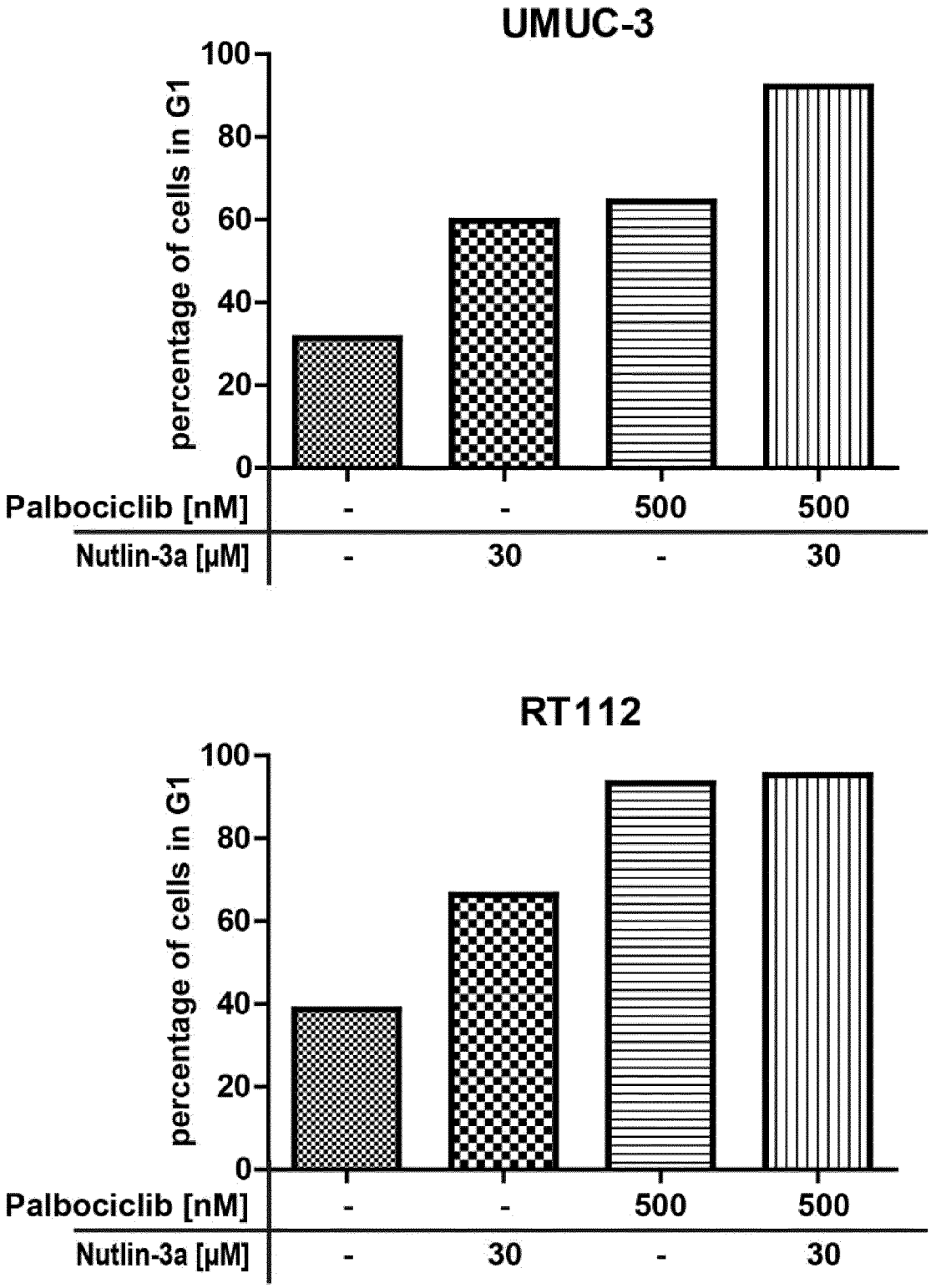

T24
percentage of cells in G1
100
80
60
40
20
0
Palbociclib [nM]
-
-
500
500
Nutlin-3a [µM]
-
30
-
30
T24shRb
percentage of cells in G1
100
80
60
40
20
0
Palbociclib [nM]
-
-
500
500
Nutlin-3a [µM]
-
30
-
30

Fig. 56 (continued)



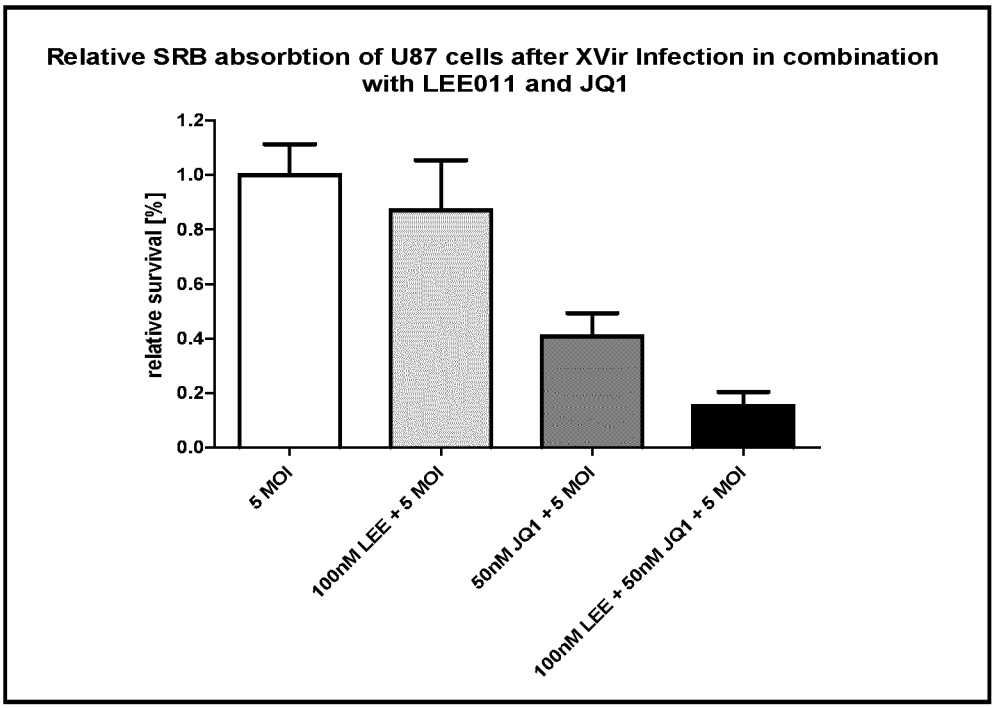

Relative SRB absorbtion of U87 cells after XVir Infection in combination with LEE011 and JQ1
relative survival [%]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
5 MOI
100nM LEE + 5 MOI
50nM JQ1 + 5 MOI
100nM LEE + 50nM JQ1 + 5 MOI

Fig. 57

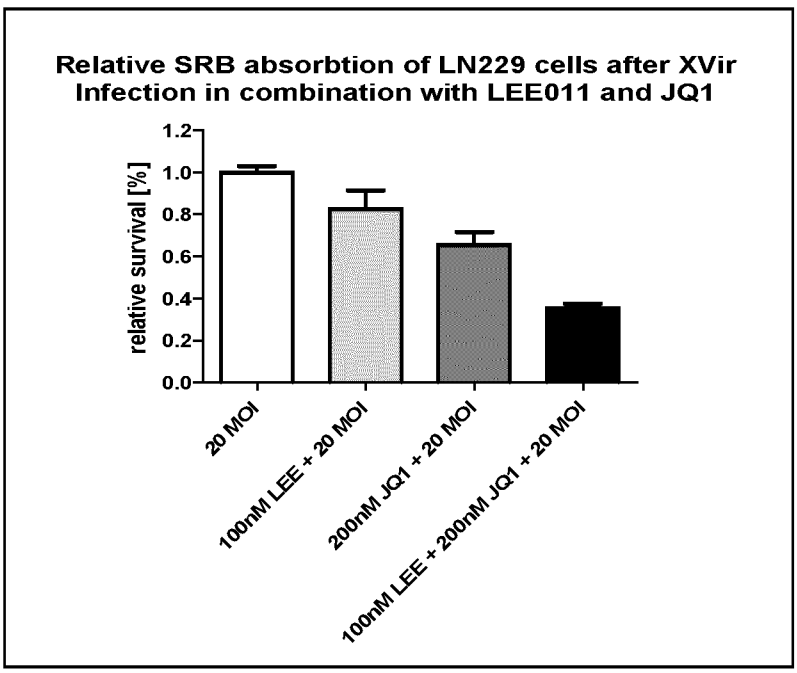

Relative SRB absorbtion of LN229 cells after XVir Infection in combination with LEE011 and JQ1
relative survival [%]
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20 MOI
100nM LEE + 20 MOI
200nM JQ1 + 20 MOI
100nM LEE + 200nM JQ1 + 20 MOI

Fig. 58

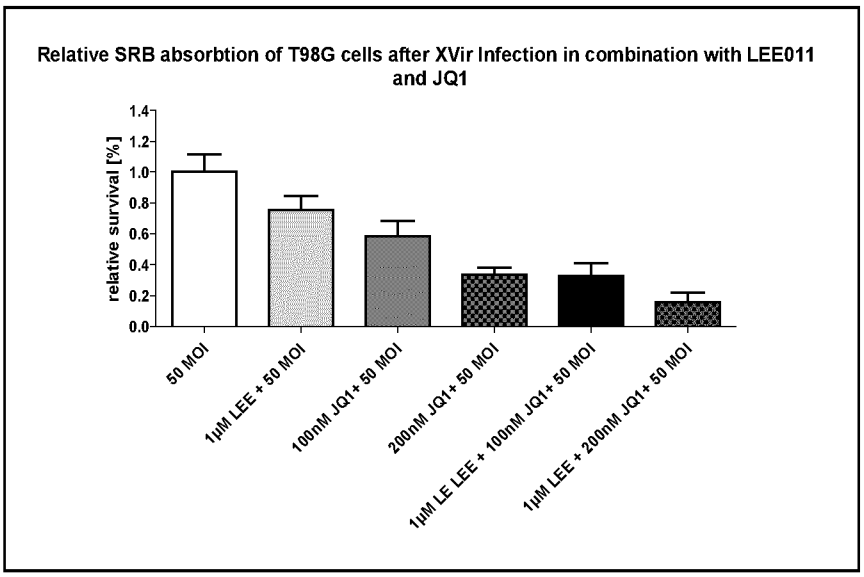

Relative SRB absorbtion of T98G cells after XVir Infection in combination with LEE011 and JQ1
relative survival [%]
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
50 MOI
1µM LEE + 50 MOI
100nM JQ1+ 50 MOI
200nM JQ1+ 50 MOI
1µM LE LEE + 100nM JQ1+ 50 MOI
1µM LEE + 200nM JQ1+ 50 MOI

Fig. 59

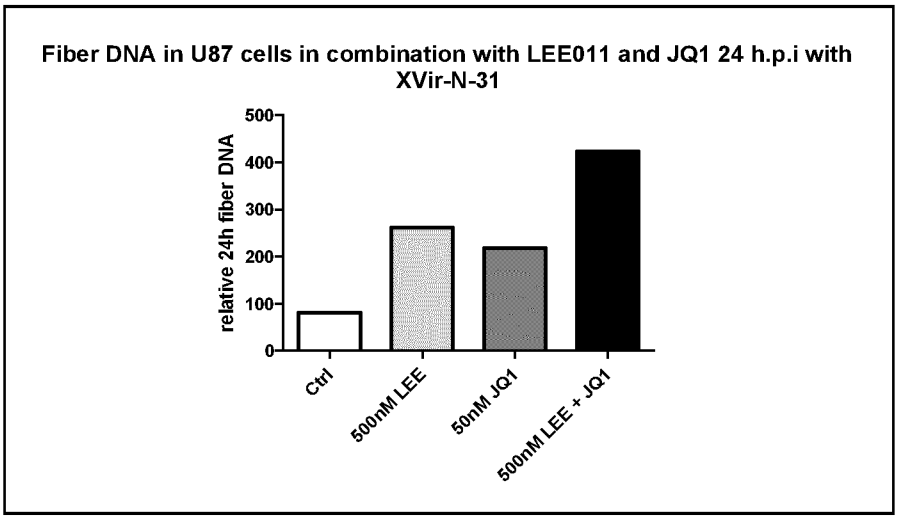

Fiber DNA in U87 cells in combination with LEE011 and JQ1 24 h.p.i with XVir-N-31
relative 24h fiber DNA
500
400
300
200
100
0
Ctrl
500nM LEE
50nM JQ1
500nM LEE + JQ1

Fig. 60

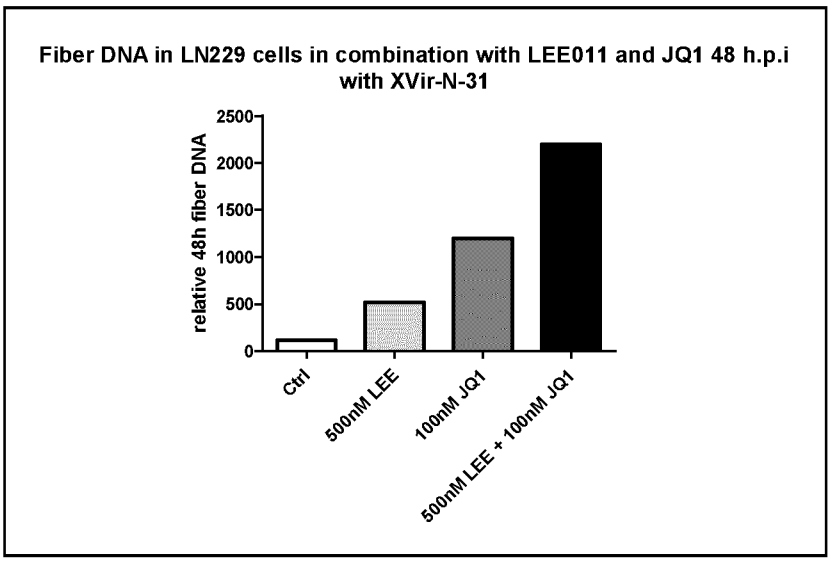

Fiber DNA in LN229 cells in combination with LEE011 and JQ1 48 h.p.i with XVir-N-31
relative 48h fiber DNA
2500
2000
1500
1000
500
0
Ctrl
500nM LEE
100nM JQ1
500nM LEE + 100nM JQ1

Fig. 61

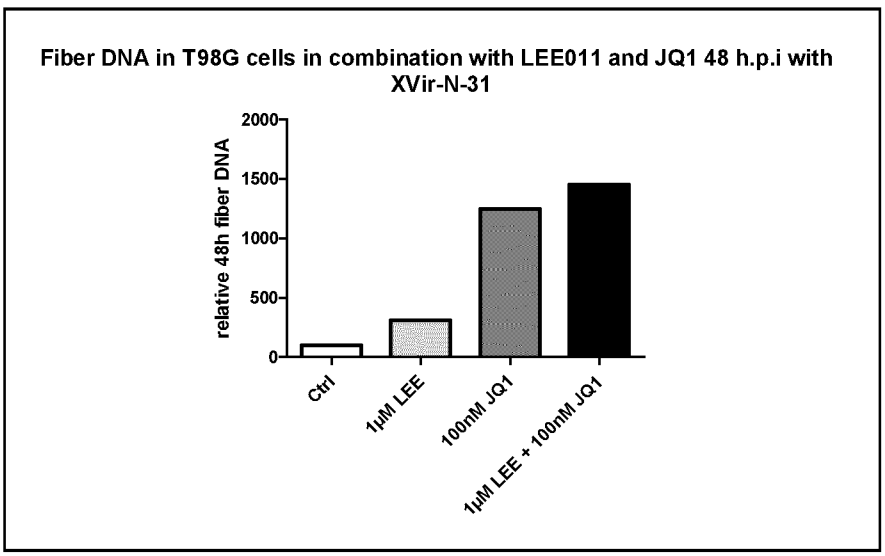

Fiber DNA in T98G cells in combination with LEE011 and JQ1 48 h.p.i with XVir-N-31
relative 48h fiber DNA
2000
1500
1000
500
0
Ctrl
1μM LEE
100nM JQ1
1μM LEE + 100nM JQ1

Fig. 62

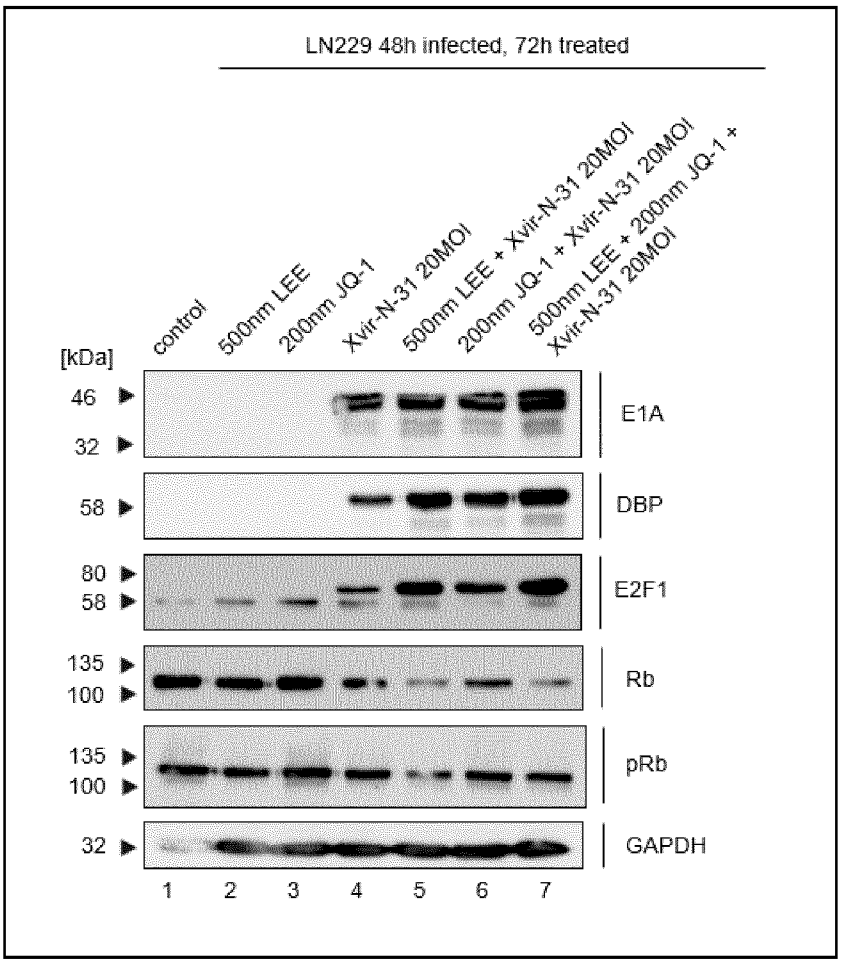

LN229 48h infected, 72h treated
control
500nm LEE
200nm JQ-1
Xvir-N-31 20MOI
500nm LEE + Xvir-N-31 20MOI
200nm JQ-1 + Xvir-N-31 20MOI
500nm LEE + 200nm JQ-1 + Xvir-N-31 20MOI
[kDa]
46
32
E1A
58
DBP
80
58
E2F1
135
100
Rb
135
100
pRb
32
GAPDH
1 2 3 4 5 6 7

Fig. 63

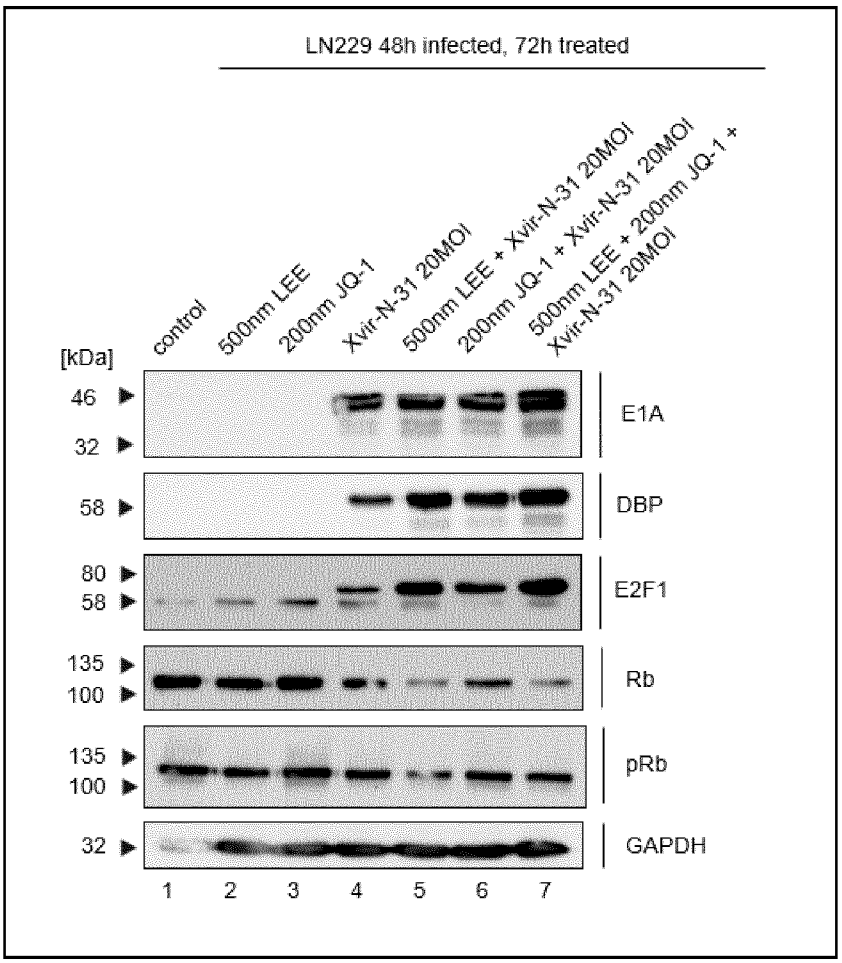

LN229 48h infected, 72h treated
control
500nm LEE
200nm JQ-1
Xvir-N-31 20MOI
500nm LEE + Xvir-N-31 20MOI
200nm JQ-1 + Xvir-N-31 20MOI
500nm LEE + 200nm JQ-1 + Xvir-N-31 20MOI
[kDa]
46
32
E1A
58
DBP
80
58
E2F1
135
100
Rb
135
100
pRb
32
GAPDH
1 2 3 4 5 6 7

Fig. 64

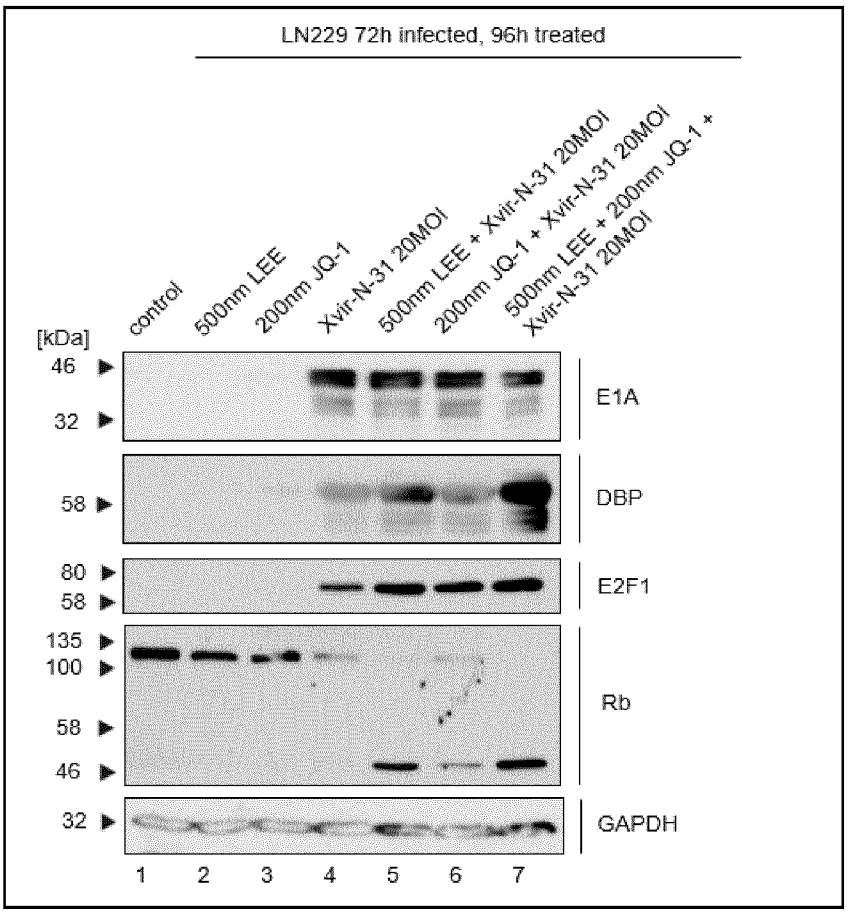

LN229 72h infected, 96h treated
control
500nm LEE
200nm JQ-1
Xvir-N-31 20MOI
500nm LEE + Xvir-N-31 20MOI
200nm JQ-1 + Xvir-N-31 20MOI
500nm LEE + 200nm JQ-1 + Xvir-N-31 20MOI
[kDa]
46
32
58
80
58
135
100
58
46
32
E1A
DBP
E2F1
Rb
GAPDH
1 2 3 4 5 6 7

Fig. 65

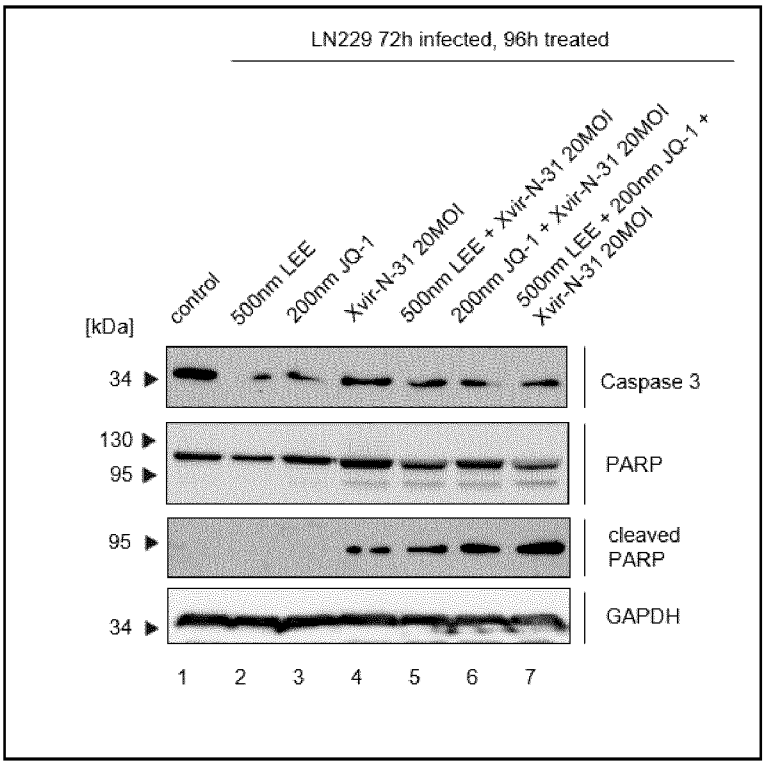

LN229 72h infected, 96h treated
control
500nm LEE
200nm JQ-1
Xvir-N-31 20MOI
500nm LEE + Xvir-N-31 20MOI
200nm JQ-1 + Xvir-N-31 20MOI
500nm LEE + 200nm JQ-1 + Xvir-N-31 20MOI
[kDa]
34
Caspase 3
130
95
PARP
95
cleaved PARP
34
GAPDH
1 2 3 4 5 6 7

Fig. 66

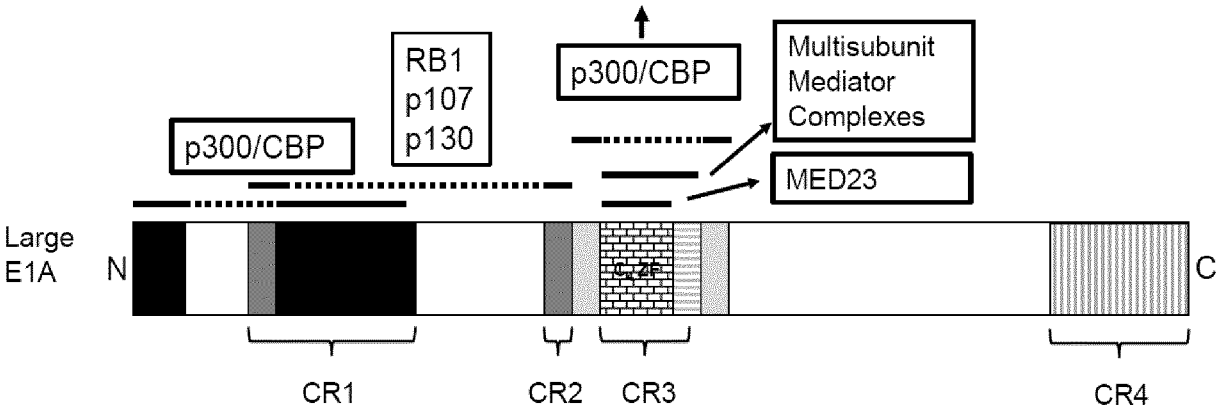
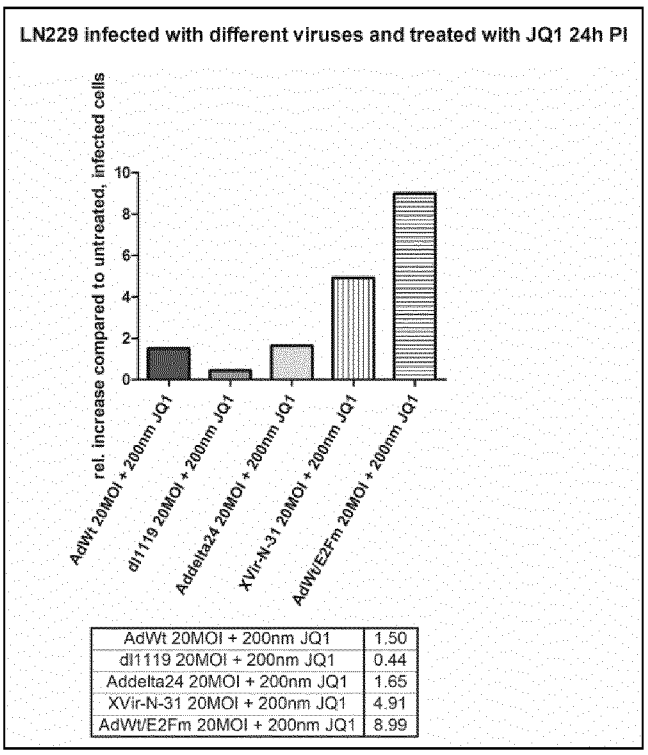
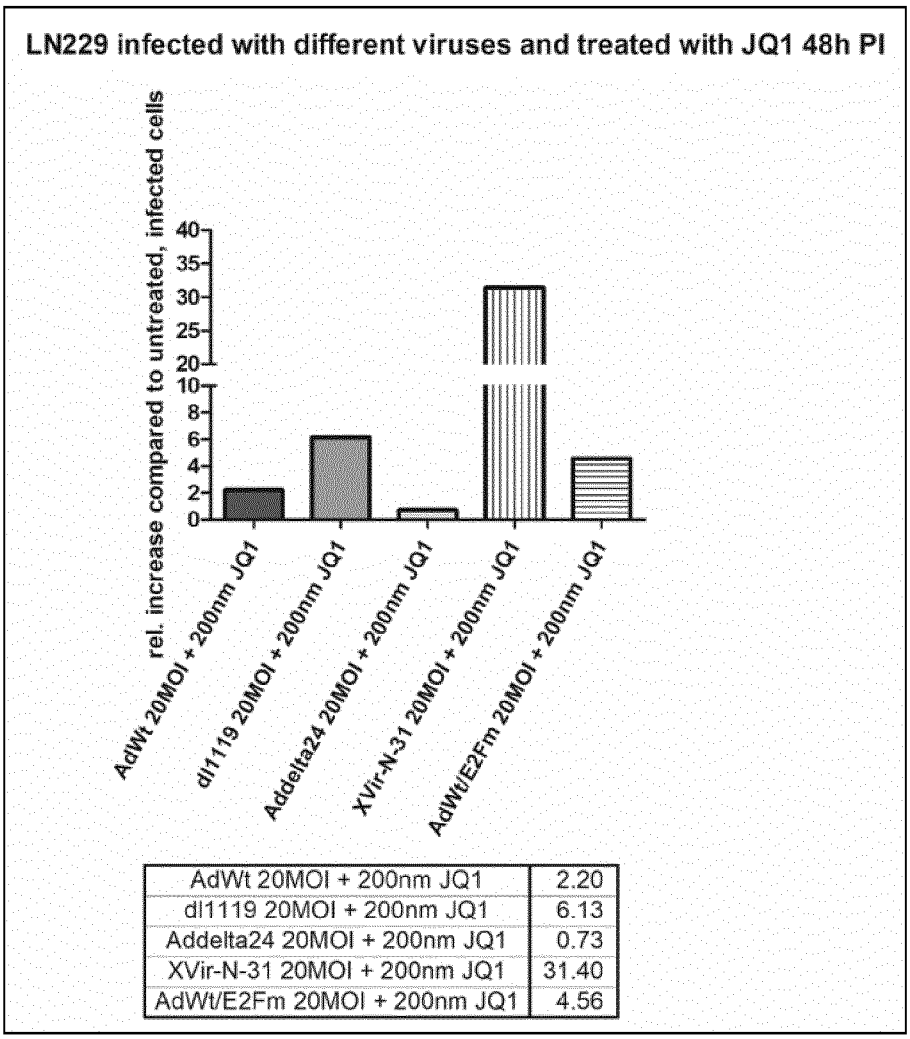

RB1
p107
p130
p300/CBP
p300/CBP
Multisubunit Mediator Complexes
MED23
Large E1A
N
C
CR1
CR2
CR3
CR4

Fig. 67



AdWt 20MOI + 200nm JQ1	1.50
dl1119 20MOI + 200nm JQ1	0.44
Addelta24 20MOI + 200nm JQ1	1.65
XVir-N-31 20MOI + 200nm JQ1	4.91
AdWt/E2Fm 20MOI + 200nm JQ1	8.99

Fig. 68



AdWt 20MOI + 200nm JQ1	2.20
dl1119 20MOI + 200nm JQ1	6.13
Addelta24 20MOI + 200nm JQ1	0.73
XVir-N-31 20MOI + 200nm JQ1	31.40
AdWt/E2Fm 20MOI + 200nm JQ1	4.56

Fig. 69

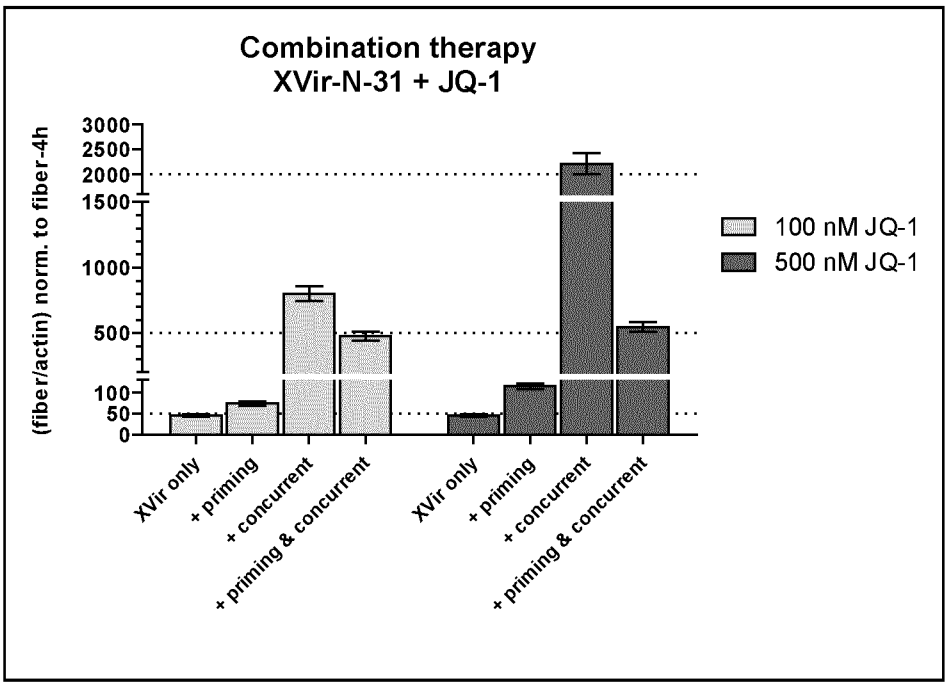

Combination therapy
XVir-N-31 + JQ-1
(fiber/actin) norm. to fiber-4h
3000
2500
2000
1500
1000
500
100
50
0
100 nM JQ-1
500 nM JQ-1
XVir only
+ priming
+ concurrent
+ priming & concurrent
XVir only
+ priming
+ concurrent
+ priming & concurrent

Fig. 70

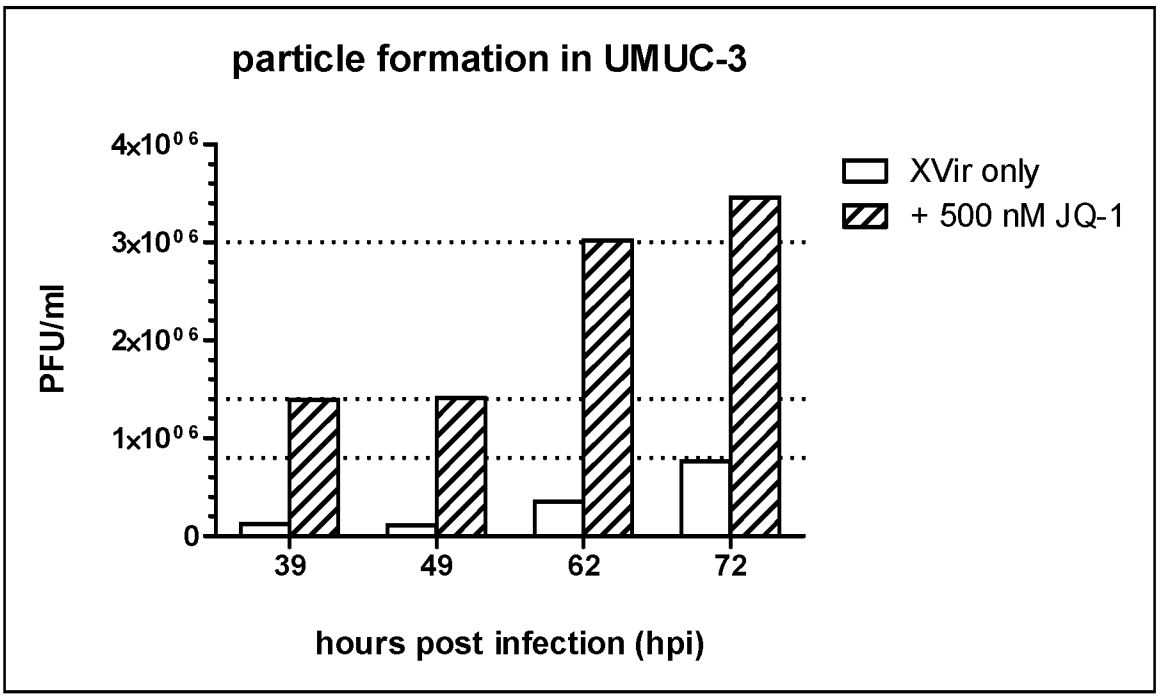

particle formation in UMUC-3
PFU/ml
4x10^06
3x10^06
2x10^06
1x10^06
0
39
49
62
72
hours post infection (hpi)
XVir only
+ 500 nM JQ-1

Fig. 71

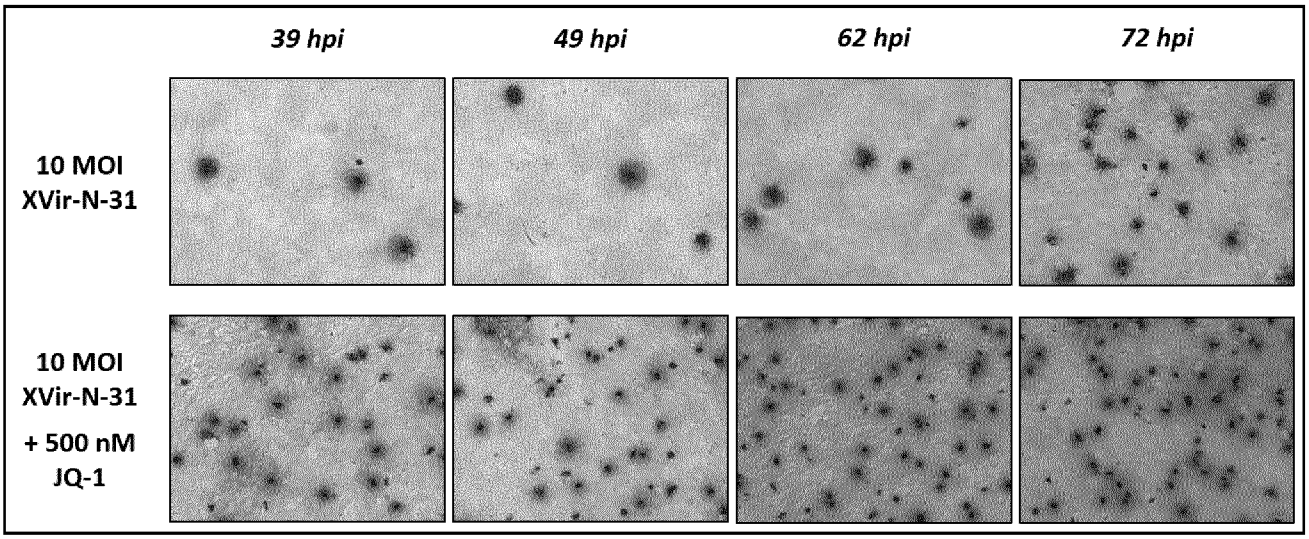

39 hpi
49 hpi
62 hpi
72 hpi
10 MOI XVir-N-31
10 MOI XVir-N-31 + 500 nM JQ-1

Fig. 72

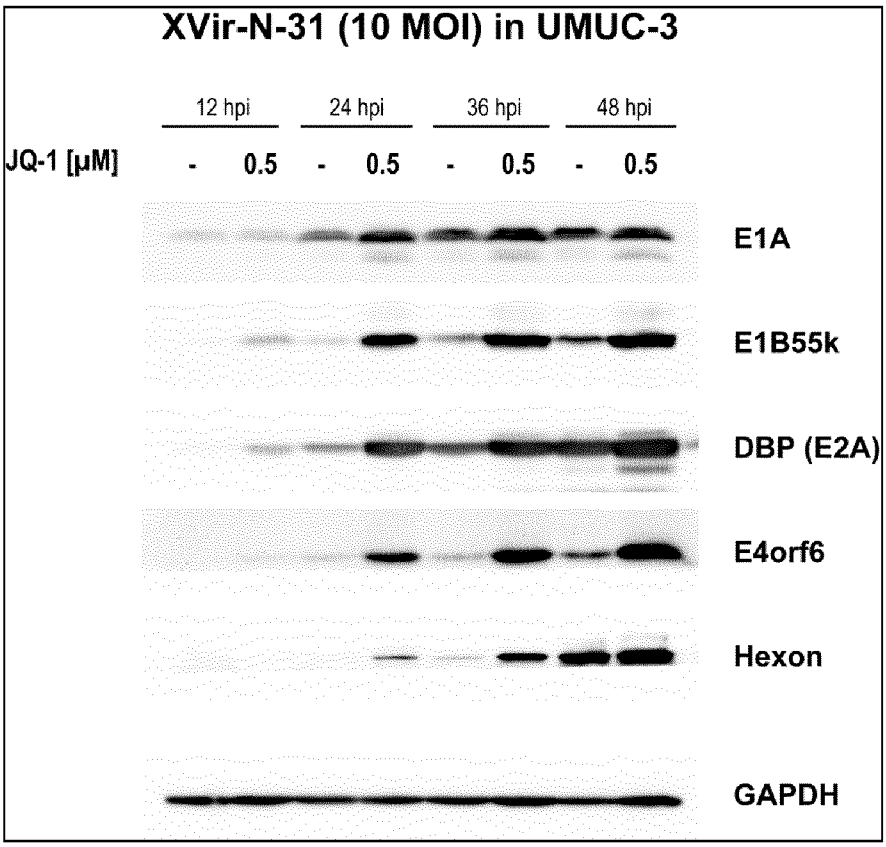

XVir-N-31 (10 MOI) in UMUC-3
12 hpi
24 hpi
36 hpi
48 hpi
JQ-1 [µM]
- 0.5 - 0.5 - 0.5 - 0.5
E1A
E1B55k
DBP (E2A)
E4orf6
Hexon
GAPDH

Fig. 73

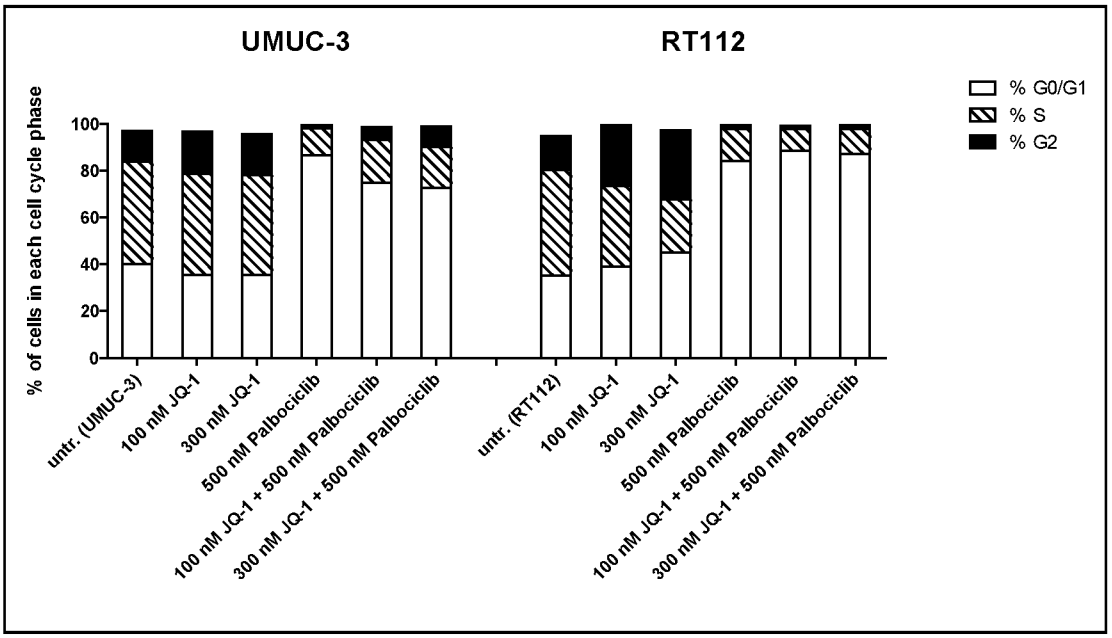

UMUC-3
RT112
% G0/G1
% S
% G2
% of cells in each cell cycle phase
100
80
60
40
20
0
untr. (UMUC-3)
100 nM JQ-1
300 nM JQ-1
500 nM Palbociclib
100 nM JQ-1 + 500 nM Palbociclib
300 nM JQ-1 + 500 nM Palbociclib
untr. (RT112)
100 nM JQ-1
300 nM JQ-1
500 nM Palbociclib
100 nM JQ-1 + 500 nM Palbociclib
300 nM JQ-1 + 500 nM Palbociclib

Fig. 74

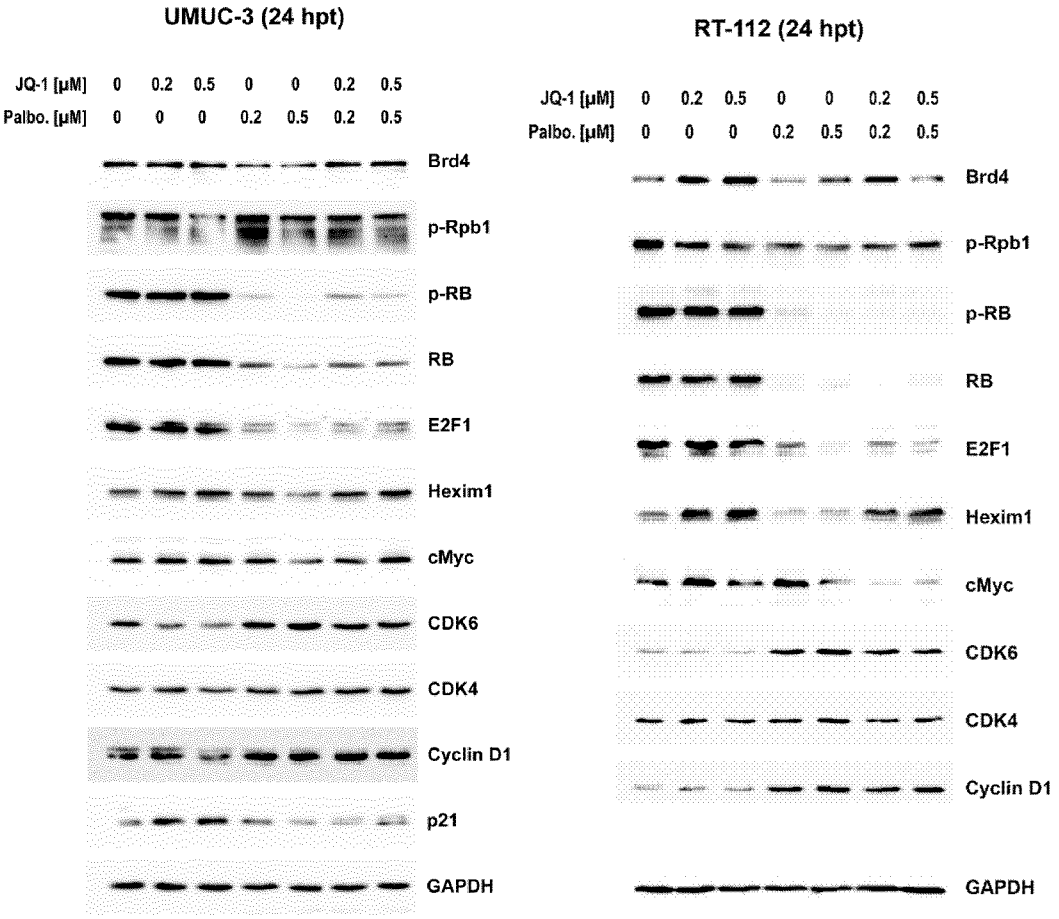

UMUC-3 (24 hpt)
JQ-1 [µM] 0 0.2 0.5 0 0 0.2 0.5
Palbo. [µM] 0 0 0 0.2 0.5 0.2 0.5
Brd4
p-Rpb1
p-RB
RB
E2F1
Hexim1
cMyc
CDK6
CDK4
Cyclin D1
p21
GAPDH
RT-112 (24 hpt)
JQ-1 [µM] 0 0.2 0.5 0 0 0.2 0.5
Palbo. [µM] 0 0 0 0.2 0.5 0.2 0.5
Brd4
p-Rpb1
p-RB
RB
E2F1
Hexim1
cMyc
CDK6
CDK4
Cyclin D1
GAPDH

Fig. 75

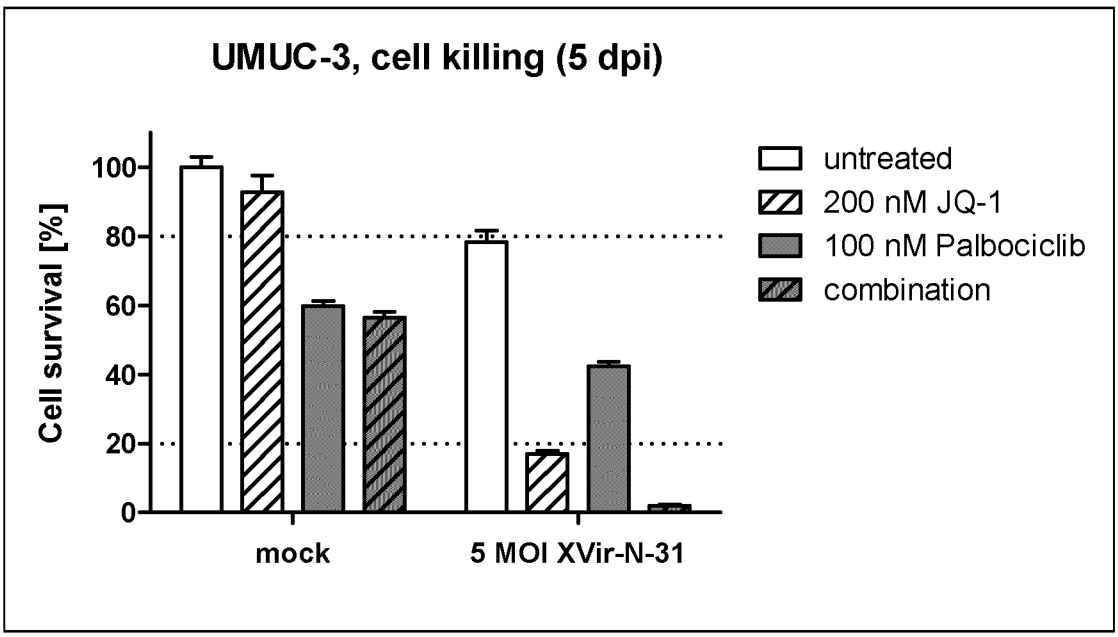

UMUC-3, cell killing (5 dpi)
Cell survival [%]
100
80
60
40
20
0
mock
5 MOI XVir-N-31
untreated
200 nM JQ-1
100 nM Palbociclib
combination

Fig. 76

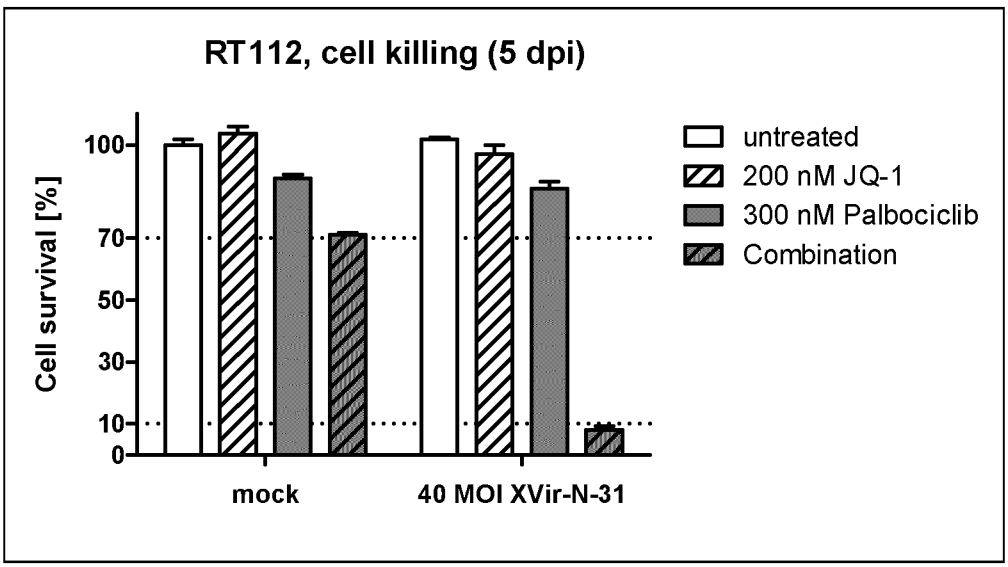

RT112, cell killing (5 dpi)
Cell survival [%]
100
70
50
30
10
0
mock
40 MOI XVir-N-31
untreated
200 nM JQ-1
300 nM Palbociclib
Combination

Fig. 77

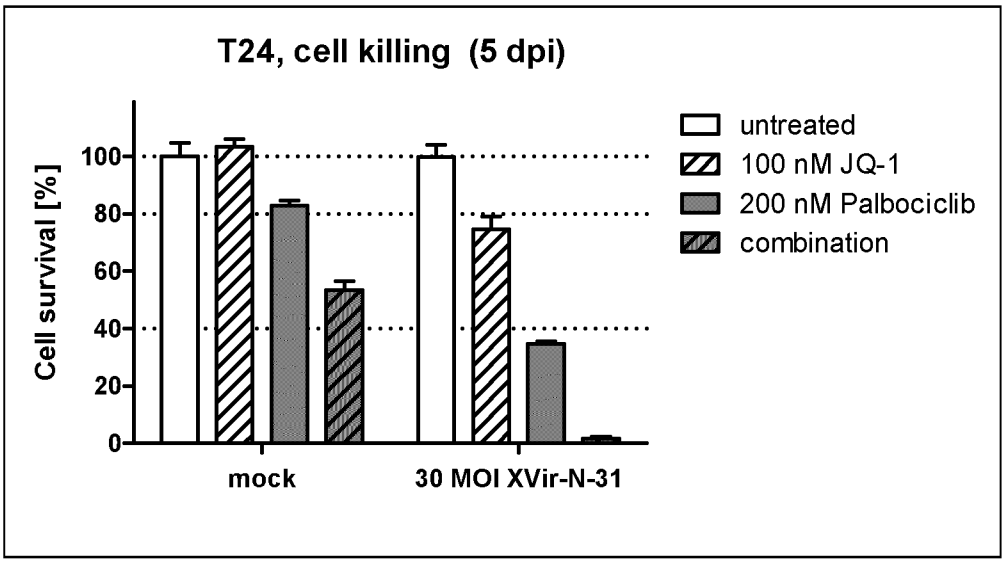

T24, cell killing (5 dpi)
Cell survival [%]
100
80
60
40
20
0
mock
30 MOI XVir-N-31
untreated
100 nM JQ-1
200 nM Palbociclib
combination

Fig. 78

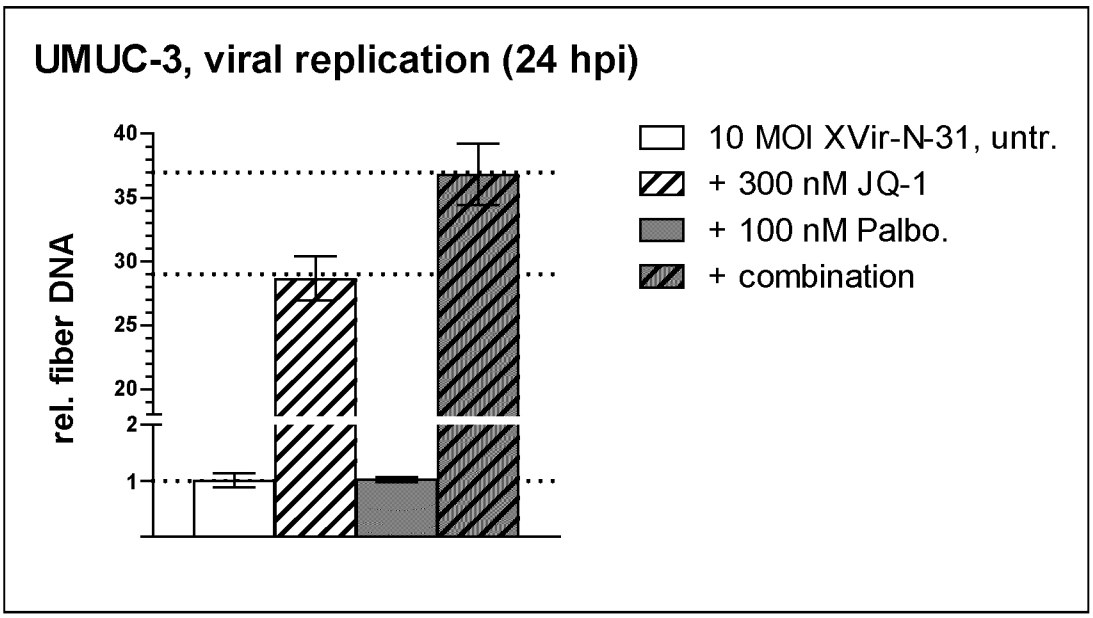

UMUC-3, viral replication (24 hpi)
rel. fiber DNA
40
35
30
25
20
2
1
10 MOI XVir-N-31, untr.
+ 300 nM JQ-1
+ 100 nM Palbo.
+ combination

Fig. 79

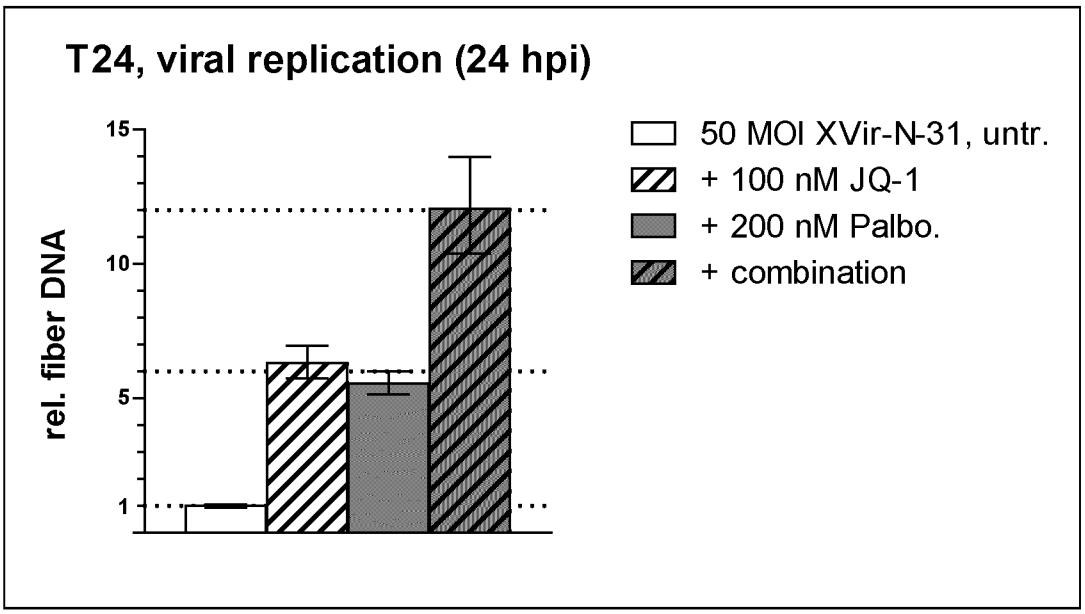

T24, viral replication (24 hpi)
rel. fiber DNA
15
10
5
1
50 MOI XVir-N-31, untr.
+ 100 nM JQ-1
+ 200 nM Palbo.
+ combination

Fig. 80

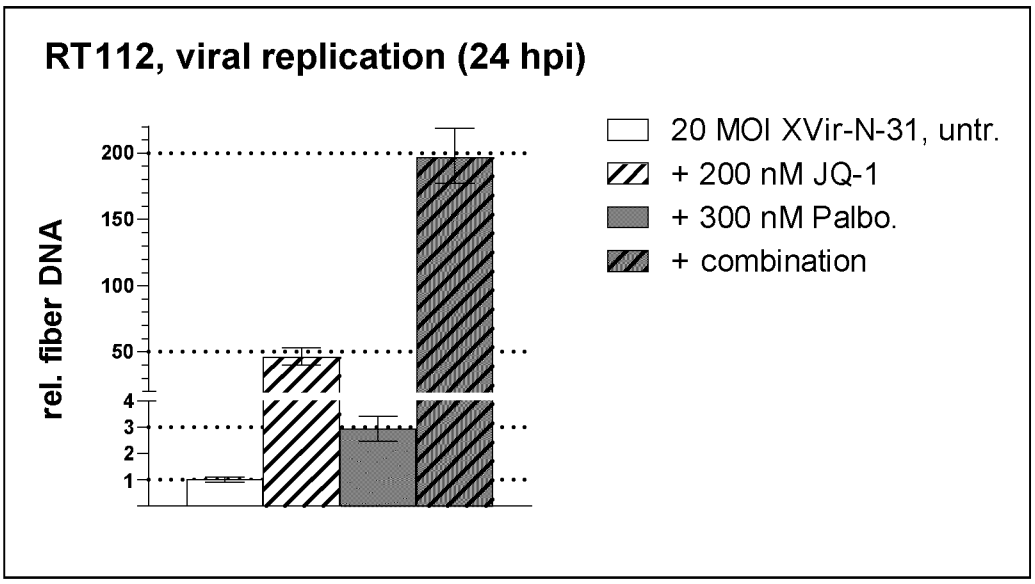

RT112, viral replication (24 hpi)
rel. fiber DNA
200
150
100
50
4
3
2
1
20 MOI XVir-N-31, untr.
+ 200 nM JQ-1
+ 300 nM Palbo.
+ combination

Fig. 81

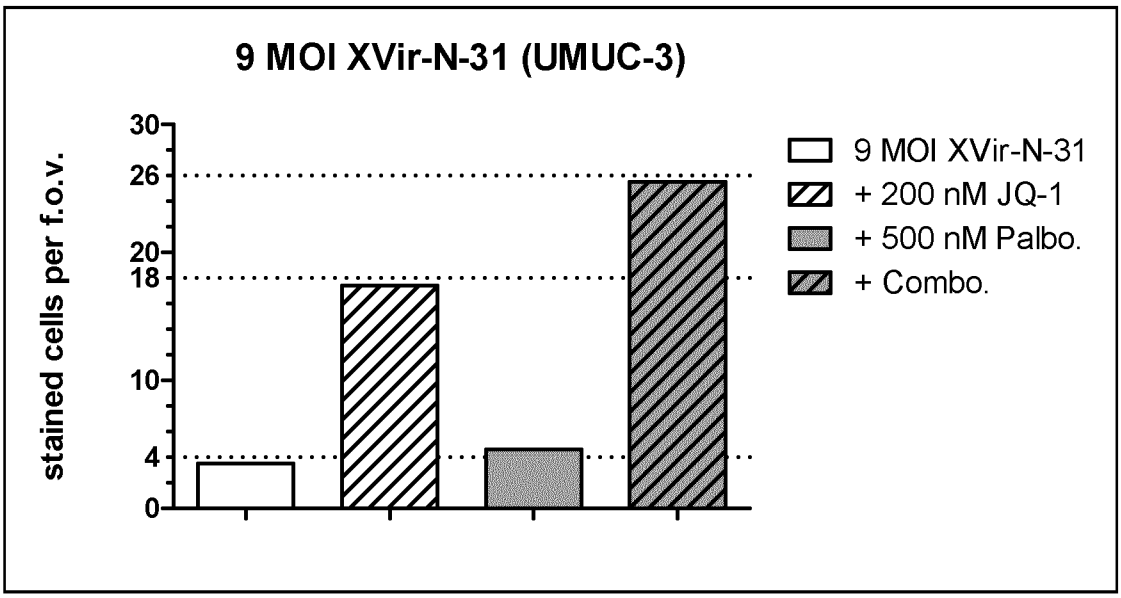

9 MOI XVir-N-31 (UMUC-3)
stained cells per f.o.v.
30
26
20
18
10
4
0
9 MOI XVir-N-31
+ 200 nM JQ-1
+ 500 nM Palbo.
+ Combo.

Fig. 82

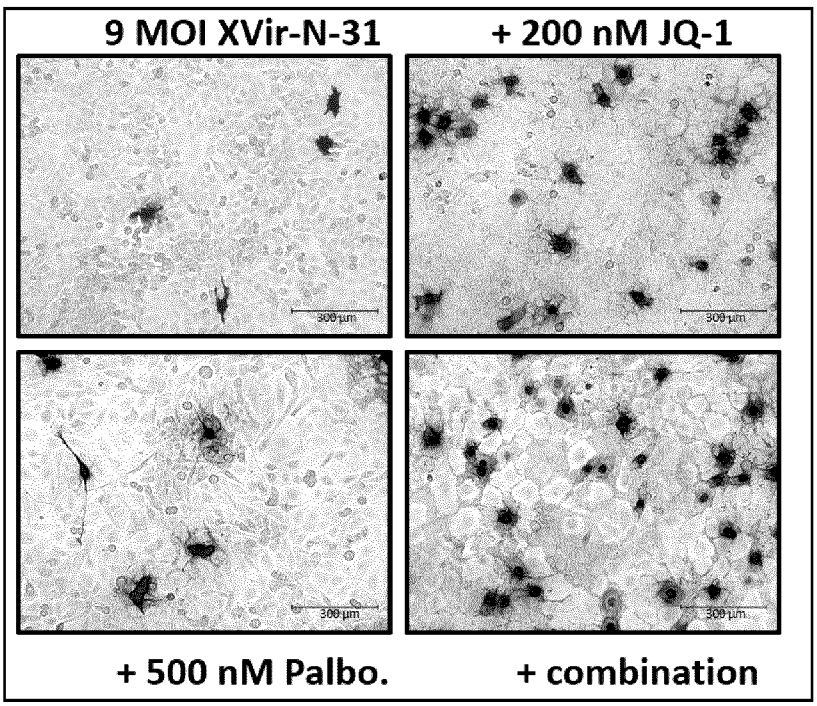

9 MOI XVir-N-31
+ 200 nM JQ-1
+ 500 nM Palbo.
+ combination

Fig. 83

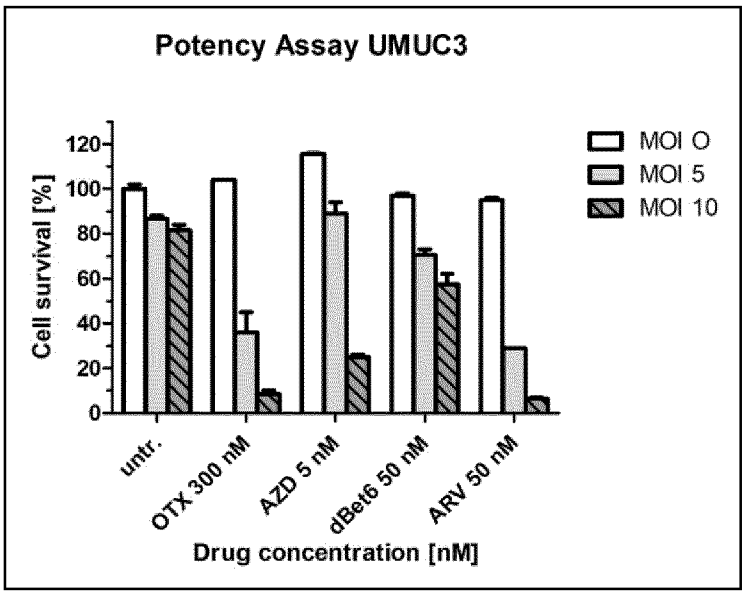

Potency Assay UMUC3
Cell survival [%]
120
100
80
60
40
20
0
MOI O
MOI 5
MOI 10
untr.
OTX 300 nM
AZD 5 nM
dBet6 50 nM
ARV 50 nM
Drug concentration [nM]

Fig. 84

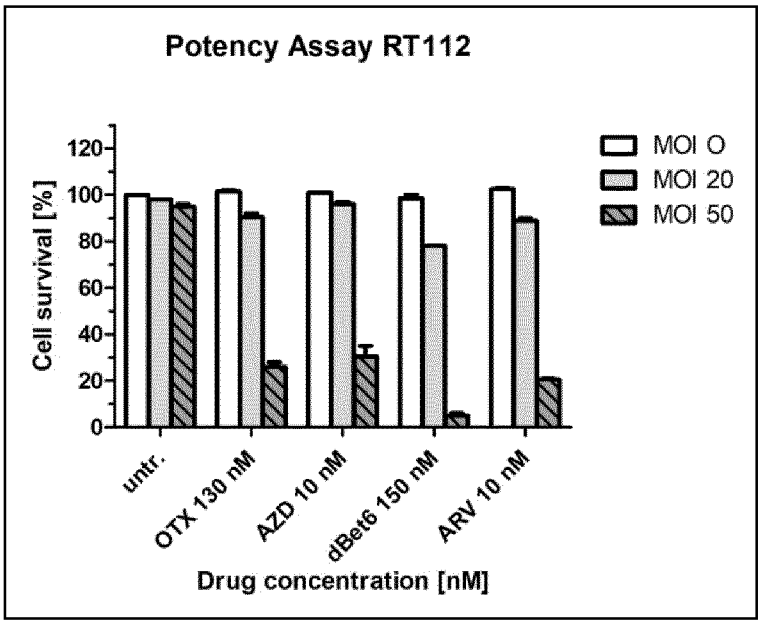

Potency Assay RT112
Cell survival [%]
120
100
80
60
40
20
0
MOI O
MOI 20
MOI 50
untr.
OTX 130 nM
AZD 10 nM
dBet6 150 nM
ARV 10 nM
Drug concentration [nM]

Fig. 85

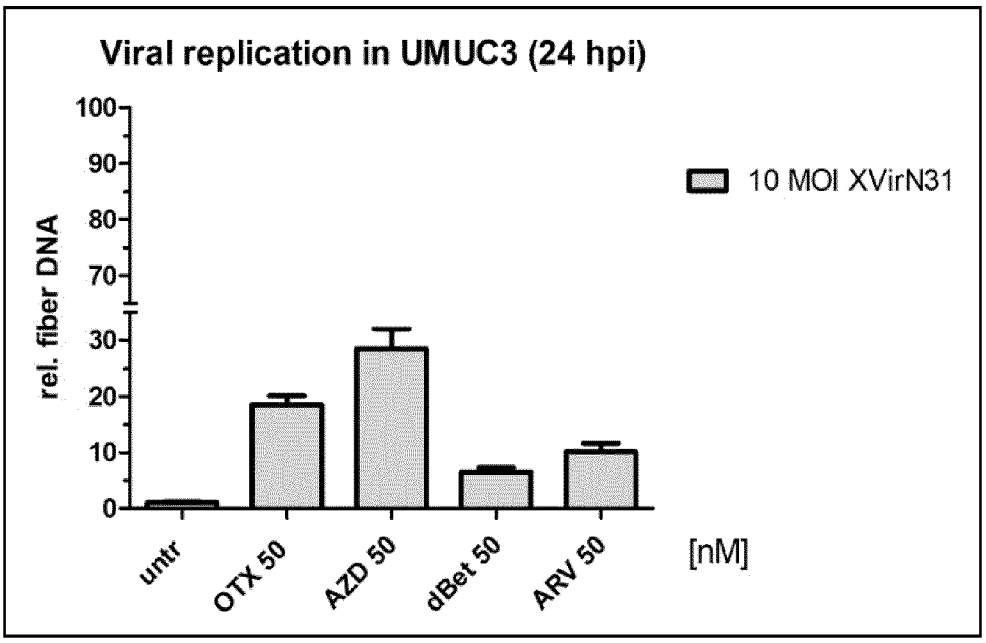

Viral replication in UMUC3 (24 hpi)
rel. fiber DNA
100
90
80
70
30
20
10
0
10 MOI XVirN31
untr
OTX 50
AZD 50
dBet 50
ARV 50
[nM]

Fig. 86

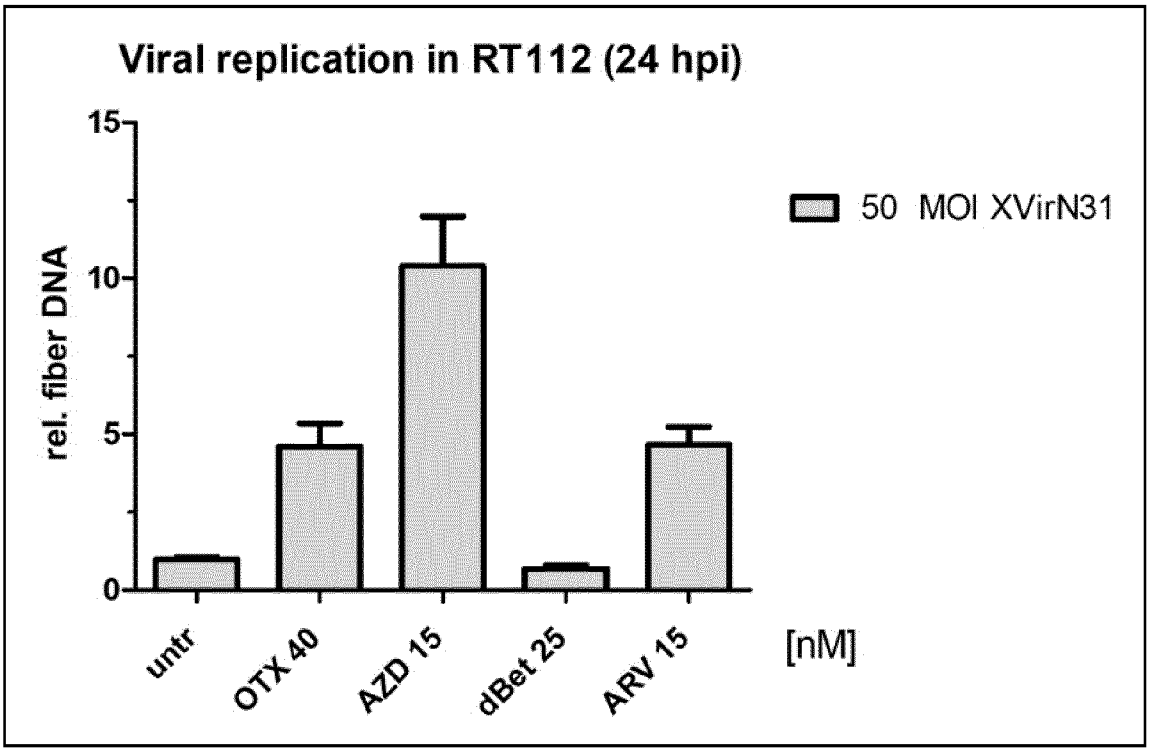

Viral replication in RT112 (24 hpi)
rel. fiber DNA
15
10
5
0
50 MOI XVirN31
untr
OTX 40
AZD 15
dBet 25
ARV 15
[nM]

Fig. 87

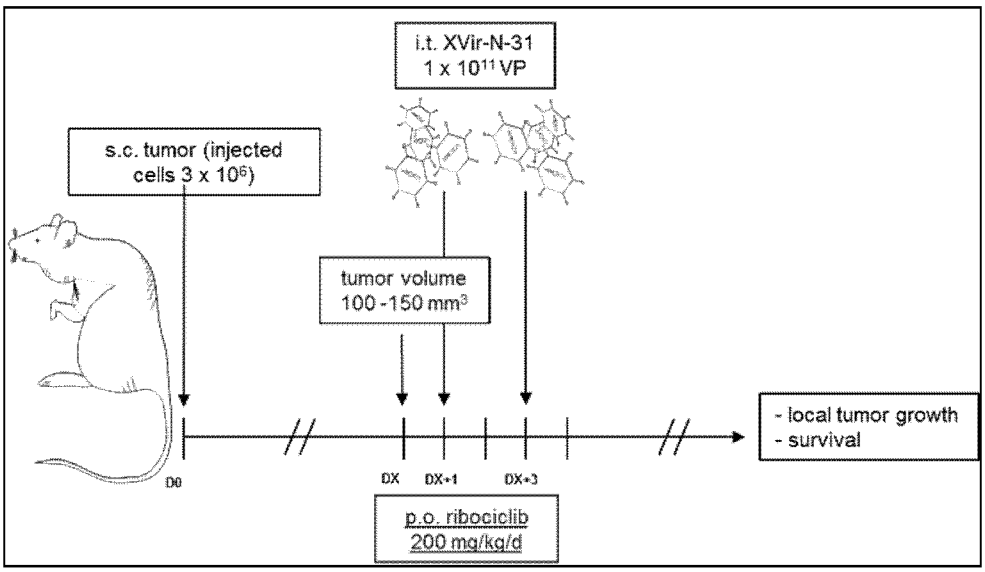

i.t. XVir-N-31
1 x 10^11 VP
s.c. tumor (injected cells 3 x 10^6)
tumor volume 100 -150 mm^3
- local tumor growth
- survival
D0
DX
DX+1
DX+3
p.o. ribociclib 200 mg/kg/d

Fig. 88

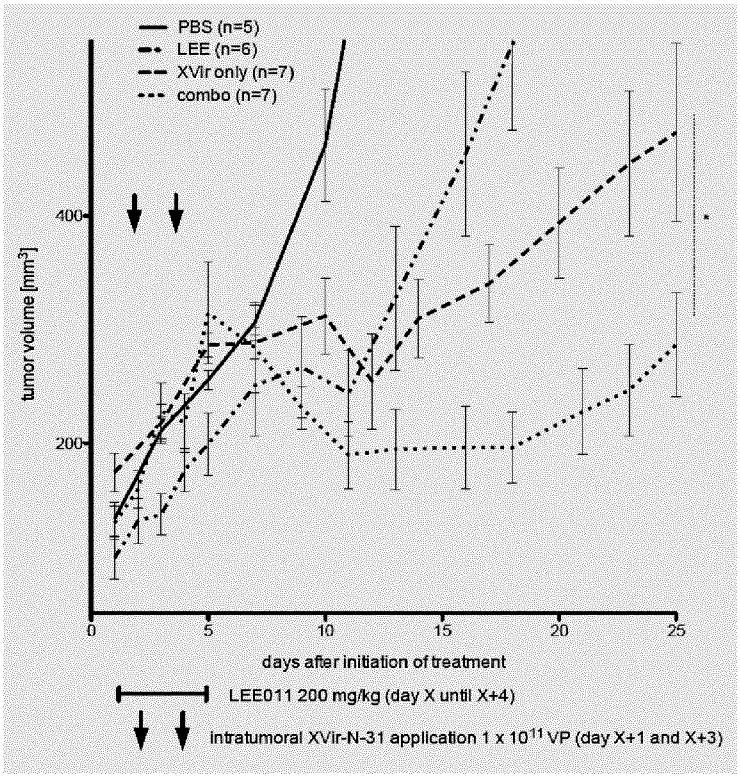

PBS (n=5)
LEE (n=6)
XVir only (n=7)
combo (n=7)
tumor volume [mm^3]
400
200
0
5
10
15
20
25
days after initiation of treatment
LEE011 200 mg/kg (day X until X+4)
intratumoral XVir-N-31 application 1 x 10^11 VP (day X+1 and X+3)

Fig. 89

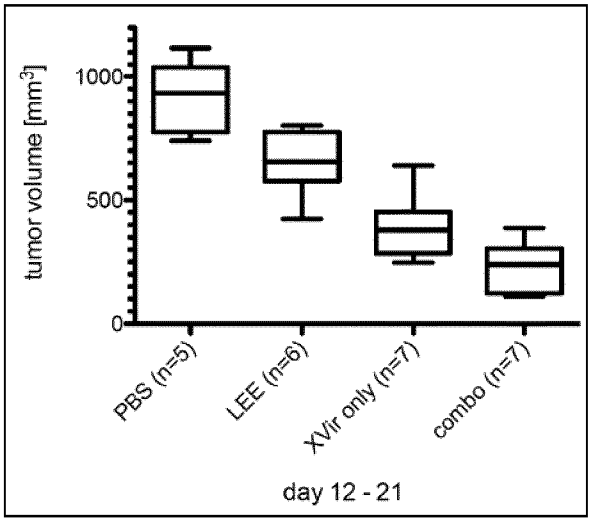

tumor volume [mm3]
1000
500
0
PBS (n=5)
LEE (n=6)
XVir only (n=7)
combo (n=7)
day 12 - 21

Fig. 90

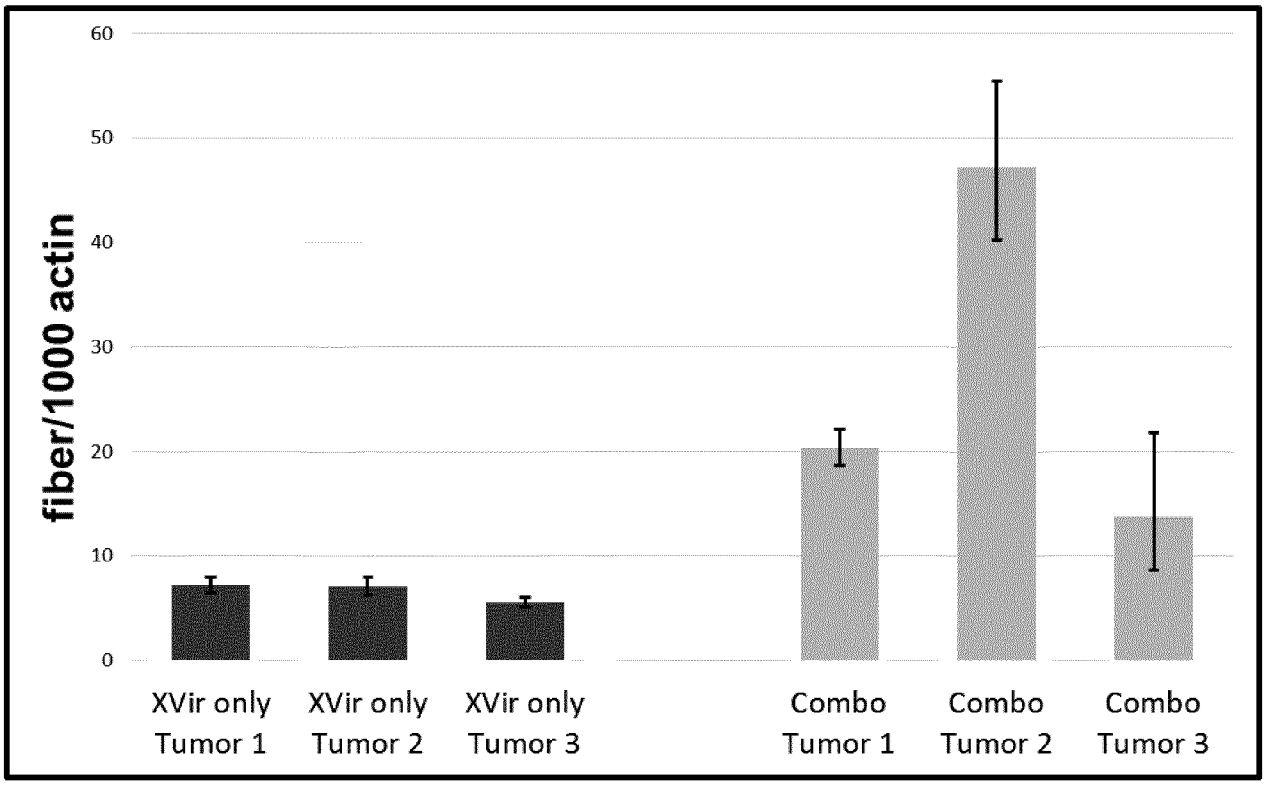

fiber/1000 actin
60
50
40
30
20
10
0
XVir only Tumor 1
XVir only Tumor 2
XVir only Tumor 3
Combo Tumor 1
Combo Tumor 2
Combo Tumor 3

Fig. 91