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**Traduzione Brevetto Europeo N. 3906970, a nome AiCuris GmbH & Co. KG, dal titolo: “PARAPOXVIRUS PER IL CONDIZIONAMENTO E IL TRATTAMENTO DI INFEZIONI DA CORONAVIRUS”**

### **DESCRIZIONE DELL'INVENZIONE**

#### CAMPO DELL'INVENZIONE

**[0001]** La presente invenzione si riferisce al trattamento di infezioni da coronavirus e alla preparazione di soggetti per una tale infezione somministrando *Parapoxvirus ovis*. Questo trattamento permette di aiutare il sistema immunitario a combattere il virus e in tal modo di prevenire e migliorare i sintomi di una malattia da coronavirus.

#### CONTESTO DELL'INVENZIONE

**[0002]** I coronavirus sono virus a RNA a singolo filamento in senso positivo, con involucro, che causano malattie nei mammiferi e negli uccelli. Negli esseri umani provocano infezioni respiratorie, compreso il comune raffreddore, anche se alcuni virus come i coronavirus SARS e MERS possono essere letali. Nuovi coronavirus emergono periodicamente in diverse aree intorno al mondo. SARS-CoV (coronavirus da sindrome respiratoria acuta grave) è comparso nel 2002 e ha causato 916 decessi durante l'epidemia, avendo un tasso di mortalità di circa il 12%. MERS-CoV (coronavirus da sindrome respiratoria del Medio Oriente) è emerso nel 2012, con 543 morti segnalati e un tasso di mortalità di circa il 39%.

**[0003]** Recentemente, un focolaio di un coronavirus denominato SARS-CoV-2 è iniziato a Wuhan, Cina, e si è diffuso da allora in tutto il mondo. La malattia causata da SARS-

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CoV-2, COVID-19 (malattia da coronavirus 2019), è stata dichiarata una pandemia dall'OMS l'11 marzo 2020. Il COVID-19 varia in gravità da asintomatico o lieve a grave, e una porzione significativa di pazienti con un'infezione clinicamente evidente sviluppa una malattia grave. Il tasso di letalità per casi varia per paese e si ritiene sia tra il 2% e il 3%, ma il tasso di mortalità reale è incerto, perché non è noto il numero totale di casi. Benché SARS-CoV-2 apparentemente abbia un tasso di mortalità inferiore a SARS-CoV e MERS-CoV, sembra essere più contagioso ed è stato in grado di diffondersi intorno al mondo, con circa 3,66 milioni di casi confermati e 257.000 decessi confermati fino al 6 maggio 2020 e presumibilmente molte altri ancora non confermati. La conoscenza del COVID-19 è incompleta e in evoluzione.

**[0004]** Le epidemie ricorrenti di coronavirus dimostrano che questi virus continuano a minacciare il mondo poiché emergono spontaneamente, si diffondono facilmente e possono avere gravi conseguenze sulla vita umana, comprese non solo le questioni sanitarie ma anche drammatiche ripercussioni sociali ed economiche. Pertanto, vi è una grande esigenza medica di prevenzione e trattamento. Tuttavia, dal momento che i coronavirus sono noti per mutare e ricombinarsi spesso, essi presentano una sfida in corso per il controllo della malattia. Attualmente non sono disponibili né vaccini né farmaci antivirali per prevenire o trattare le infezioni da coronavirus umano. In tutto il mondo si sta sperimentando un gran numero di antivirali per un effetto sul coronavirus, con candidati promettenti che non hanno avuto successo.

**[0005]** Inaspettatamente, gli inventori hanno trovato che *Parapoxvirus ovis* (PPVO) è utile per preparare i soggetti e trattare le infezioni da coronavirus. PPVO causa delle infezioni

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cutanee acute nella capra e nella pecora e, pur non portando a malattie gravi nell'uomo, induce una complessa risposta immunitaria innata e adattativa nell'uomo e tratta infezioni con alcuni virus specifici come HBV (WO 2019/048640 A1). Tuttavia, la risposta immunitaria indotta da PPVO è complessa e non necessariamente utile per trattare le infezioni virali in generale. Pertanto, non ci si sarebbe potuto attendere che PPVO avesse utilità nel preparare i soggetti e trattare le infezioni da coronavirus.

#### RIASSUNTO DELL'INVENZIONE

**[0006]** In un primo aspetto, la presente invenzione si riferisce a un agente *Parapoxvirus ovis* (PPVO) per l'uso nel (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto. In un secondo aspetto, l'invenzione si riferisce a una composizione o un kit comprendente un agente *Parapoxvirus ovis* (PPVO) e un agente coronavirus. In un terzo aspetto, la presente invenzione si riferisce a una composizione farmaceutica o un kit comprendente un agente *Parapoxvirus ovis* (PPVO) per l'uso medico secondo l'invenzione, la composizione farmaceutica o il kit comprendendo un agente PPVO e preferibilmente un ulteriore medicamento.

#### LEGENDE ALLE FIGURE

**[0007]**

**Figura 1: Stimolazione di attività antivirale contro SARS-CoV-2 indotta da parapoxvirus inattivato (iPPVO) che è stata potenziata da approcci combinatoriali.**

Culture di sangue intero sono state stimulate con iPPVO +/- 5 µg/ml di ConA. Quando non si aggiungeva ConA, è stato invece aggiunto PBS. Il veicolo di iPPVO +/- ConA o PBS +/- ConA, rispettivamente, sono serviti come controllo. ConA 5 µl/ml è stato

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testato come un co-stimolo. Le colture sono state incubate per 3 giorni e sono stati raccolti i supernatanti. Il supernatante è stato trasferito su cellule Vero-eGFP che 2-4 ore dopo sono state infettate con SARS-CoV-2 per determinare l'attività antivirale dei rispettivi campioni di surnatante e incubate per 5 giorni. La fluorescenza eGFP come misura per le cellule viventi è stata analizzata usando il software ImageJ. La media del controllo cellulare in cui non è stato aggiunto il virus è servita come controllo positivo (media CC) ed è stata fissata al 100% (si veda la linea tratteggiata superiore). Le cellule infettate con SARS-CoV2 ma non trattate hanno dato la più alta distruzione possibile di cellule viventi e sono servite come controllo negativo indicato come controllo medio del virus (media VC). L'attività antivirale dei campioni è stata calcolata rispetto al controllo positivo media CC e indicata in %.

**Figura 2: iPPVO D1701 e iPPVO NZ2 stimolano l'attività antivirale contro SARS-CoV-2.** Colture di sangue intero sono state stimolate con iPPVO. Il veicolo di iPPVO fungeva da controllo negativo. iPPVO D1701 fungeva da controllo positivo. Le colture sono state incubate per 3 giorni e sono stati raccolti i surnatanti. Il surnatante è stato trasferito su cellule Vero-eGFP che 2-4 ore dopo sono state infettate con SARS-CoV-2 per determinare l'attività antivirale residente nei rispettivi campioni di surnatante e incubate per 5 giorni. La fluorescenza di eGFP come misura per le cellule viventi che sono state protette contro SARS-CoV-2 per l'attività antivirale delle citochine nel rispettivo surnatante è stata misurata usando il software ImageJ. L'attività antivirale del controllo positivo è stata fissata al 100% (si veda la linea tratteggiata superiore). L'attività antivirale dei campioni è stata calcolata rispetto al controllo positivo e indicata

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in %. La linea tratteggiata inferiore segna il valore del controllo negativo.

#### DESCRIZIONE DETTAGLIATA DELL'INVENZIONE

**[0008]** Prima che la presente invenzione sia descritta in dettaglio di seguito, si deve comprendere che questa invenzione non è limitata alla metodologia, ai protocolli e ai reagenti particolari qui descritti poiché questi possono variare. Si deve comprendere anche che la terminologia qui usata ha lo scopo di descrivere solo particolari forme di realizzazione, e non è intesa per limitare l'ambito della presente invenzione che sarà limitato solo dalle rivendicazioni allegate. Se non definiti altrimenti, tutti i termini tecnici e scientifici usati in questo testo hanno lo stesso significato comunemente compreso da un esperto ordinario del ramo.

**[0009]** Preferibilmente, i termini qui usati sono definiti come descritto in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", Leuenberger, H.G.W, Nagel, B. and Kölbl, H. eds. (1995), Helvetica Chimica Acta, CH-4010 Basel, Switzerland).

**[0010]** Nel seguito, saranno descritti gli elementi della presente invenzione. Questi elementi sono elencati con forme di realizzazione specifiche, tuttavia si dovrebbe comprendere che essi possono essere combinati in qualsiasi modo e in qualsiasi numero per creare delle forme di realizzazione aggiuntive. Gli esempi variamente descritti e le forme di realizzazione preferite non dovrebbero essere intesi per limitare la presente invenzione alle sole forme di realizzazione esplicitamente descritte. Questa descrizione dovrebbe essere intesa per supportare e annoverare le forme di realizzazione che combinano le forme di realizzazione descritte esplicitamente con un numero qualsiasi degli elementi esposti e/o preferiti. Inoltre, qualsiasi permutazione e combinazione di tutti gli

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elementi descritti in questa domanda dovrebbe essere considerata esposta dalla descrizione della presente domanda a meno che il contesto non indichi altrimenti.

**[0011]** In tutta questa specifica e nelle rivendicazioni che seguono, a meno che il contesto richieda altrimenti, la parola “comprendere”, e variazioni quali “comprende” e “comprendente”, sono da intendersi per implicare l’inclusione di un numero intero o passaggio o gruppo di numeri interi o passaggi affermati ma non l’esclusione di qualsiasi altro numero intero o passaggio o gruppo di numeri interi o passaggi. Nelle forme di realizzazione preferite, “comprendere” può significare “consistere in”. Nell’uso fatto in questa specifica e nelle rivendicazioni allegate, le forme singolari “un”, “uno” e “il” includono i referenti plurali, a meno che il contenuto non imponga chiaramente altrimenti.

**[0012]** In un primo aspetto, la presente invenzione si riferisce a un agente *Parapoxvirus ovis* (PPVO) per l'uso nel (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto.

*Agente parapoxvirus*

**[0013]** Il parapoxvirus è *Parapoxvirus ovis* (PPVO). Questo include qualsiasi ceppo di PPVO, preferibilmente qualsiasi ceppo della specie PPVO classificato dal Comitato Internazionale sulla Tassonomia dei Virus (*International Committee on Taxonomy of Viruses*, ICTV). Ceppi esemplificativi sono NZ2, NZ7, NZ10, D1701, OV/20, OV/7, OV/C2, OV/mi-90, OV-Torino, SAOO, Bo29, orf11, ceppo orf Greco 155 e ceppo orf Greco 176. I ceppi preferiti sono NZ2 e D1701, specialmente NZ2.

**[0014]** L'agente PPVO è, in una forma di realizzazione preferita,

(a) il PPVO stesso o un frammento di esso, o

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(b) un agente che comprende delle informazioni genetiche codificanti per (a).

**[0015]** In una forma di realizzazione più preferita, è

- (i) un virione di PPVO vivo,
- (ii) un virione di PPVO inattivato,
- (iii) un frammento di (i) o (ii),
- (iv) un acido nucleico codificante per qualsiasi da (i) a (iii),
- (v) un vettore comprendente l'acido nucleico di (iv), o
- (vi) una cellula comprendente l'acido nucleico di (iv) o il vettore di (v).

**[0016]** Il virione di PPVO vivo può essere o non essere attenuato. I virioni di PPVO attenuati sono noti nella tecnica, ad es. virioni privi di un gene di virulenza come vegf-e e/o gif. Il frammento può essere riconosciuto dal sistema immunitario e preferibilmente stimola una risposta immunitaria (ossia è un antigene e preferibilmente un immunogeno) e può essere qualsiasi frammento, sebbene preferibilmente sia un frammento che è legato da un recettore di riconoscimento dei profili (PRR) umano, ad es. TLR9. In una forma di realizzazione, il frammento di PPVO è una proteina di PPVO.

**[0017]** I frammenti esemplificativi sono (sequenza codificante esemplificativa (posizioni nucleotidiche di SEQ ID NO: 3 che rappresenta il ceppo NZ2) tra parentesi che precede il frammento): (da 3 a 539) ORF L1, (da 781 a 449) ORF L2r, (da 1933 a 1664) ORF L3r, (da 3269 a 2790) ORF L4r, (da 2799 a 3851) ORF L5, (da 2962 a 3753) ORF L6, (da 3784 a 3122) ORF L7r, (da 4341 a 4129) ORF L8r, (da 4904 a 4428) ORF 1ar, (da 6517 a 4970) ORF 1r, (da 8042 a 6684) ORF 2r, (da 9989 a 8070) ORF 3r, (da 11195 a 10062) ORF 4r, (da 11493 a 11227) ORF 5r, (da 11802 a 12038) ORF 6, (da 12358 a 12080) ORF 7r,

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(da 13980 a 12364) ORF 8r, (da 14826 a 14053) ORF 9ar, (da 15080 a 15394) ORF 10, (da 16838 a 15423) ORF 11r, (da 19021 a 16847) ORF 12r, (da 19704 a 19156) ORF 13r, (da 20314 a 19736) ORF 14r, (da 20401 a 22101) ORF 15, (da 22125 a 22940) ORF 6, (da 23003 a 23866) ORF 17, (da 26908 a 23873) ORF 18r, (da 26926 a 27213) ORF 19, (da 27626 a 27216) ORF 20r, (da 29754 a 27616) ORF 21r, (da 32217 a 29800) ORF 22r, (da 33380 a 32418) ORF 23r, (da 33602 a 33393) ORF 24r, (da 34466 a 33612) ORF 25r, (da 34735 a 34502) ORF 26r, (da 35905 a 34739) ORF 27r, (da 37194 a 35905) ORF 28r, (da 37200 a 39248) ORF 29; 41037 a 39229) ORF 30r, (da 41374 a 42066) ORF 31, (da 42336 a 41731) ORF 32r, (da 42407 a 41997) ORF 33r, (da 42410 a 43765) ORF 34, (da 43770 a 43958) ORF 35, (da 43980 a 44534) ORF 36, (da 45727 a 44537) ORF 37r, (da 45760 a 46557) ORF 38, (da 46567 a 47568) ORF 39, (da 47572 a 48303) ORF 40, (da 48352 a 48621) ORF 41, (da 49887 a 48634) ORF 42r, (da 49917 a 50793) ORF 43, (da 50719 a 51102) ORF 44, (da 51059 a 51511) ORF 44a, (da 51584 a 52591) ORF 45, (da 52509 a 53066) ORF 46, (da 53523 a 53023) ORF 47r, (da 53607 a 57473) ORF 48, (da 58070 a 57528) ORF 49r, (da 57700 a 58662) ORF 50, (da 59674 a 58673) ORF 51r, (da 62089 a 59678) ORF 52r, (da 62198 a 62881) ORF 53, (da 62909 a 63862) ORF 55, (da 63858 a 64271) ORF 56, (da 64309 a 66831) ORF 57, (da 67266 a 66799) ORF 58r, (da 67803 a 67273) ORF 58ar, (da 67915 a 68607) ORF 59, (da 68624 a 70984) ORF 60, (da 70994 a 72898) ORF 61, (da 72938 a 73507) ORF 62, (da 73540 a 74211) ORF 63, (da 76120 a 74207) ORF 64r, (da 76749 a 76186) ORF 65r, (da 77698 a 76799) ORF 66r, (da 79343 a 77709) ORF 67r, (da 79816 a 79367) ORF 68r, (da 80529 a 79858) ORF 69r, (da 80774 a 80529) ORF 70r, (da 82815 a 80788) ORF 71r, (da 83835 a 82834) ORF 72r,

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(da 83874 a 85583) ORF 73, (da 85535 a 84402) ORF 74r, (da 88096 a 85574) ORF 75r,  
(da 87759 a 88667) ORF 76, (da 88920 a 88642) ORF 77r, (da 91652 a 88938) ORF 78r,  
(da 91667 a 92674) ORF 79, (da 93466 a 92681) ORF 80r, (da 93761 a 93486) ORF 81r,  
(da 94060 a 93788) ORF 82r, (da 94238 a 94080) ORF 83r, (da 94508 a 94242) ORF 84r,  
(da 95571 a 94498) ORF 85r, (da 96187 a 95600) ORF 86r, (da 96202 a 97665) ORF 87,  
(da 97915 a 97643) ORF 88r, (da 98251 a 99537) ORF 89, (da 99537 a 99974) ORF 90,  
(da 100001 a 101140) ORF 91, (da 101168 a 104650) ORF 92, (da 106354 a 104795) ORF  
93r, (da 107947 a 106400) ORF 94r, (da 108256 a 107990) ORF 95r, (da 108719 a 108300)  
ORF 96r, (da 109679 a 108738) ORF 97r, (da 109861 a 109682) ORF 98r, (da 110830 a  
10033) ORF 99r, (da 110208 a 110417) ORF 100, (da 110469 a 110651) ORF 100a,  
(da 110915 a 111397) ORF 101, (da 111419 a 111913) ORF 102, (da 111949 a 112485)  
ORF 103, (da 112593 a 113450) ORF 104, (da 113323 a 112967) ORF 105r, (da 113526 a  
114152) ORF 106, (da 114199- a 115236) ORF 107, (da 115353 a 115787) ORF 108,  
(da 115859 o 116551) ORF 109, (da 116729 a 117523) ORF 110, (da 117572 a 117114)  
ORF 111r, (da 117423 a 118085) ORF 112, (da 118968 a 118375) ORF 114r, (da 118508 a  
119119) ORF 115, (da 119588 a 120202) ORF 116, (da 120314 a 21231) ORF 117,  
(da 121380 a 123920) ORF 118, (da 121288 a 122256) ORF 119, (da 122350 a 123924)  
ORF 120, (da 123962 a 125566) ORF 121, (da 125193 a 124591) ORF 122r, (da 125689  
a 123935) ORF 123r, (da 123839 a 123297) ORF 123ar, (da 125652 a 126170) ORF 124,  
(da 126121 a 125699) ORF 125r, (da 126279 a 127769) ORF 126, (da 127851 a 128408)  
ORF 127, (da 128520 a 130076) ORF 128, (da 130105 a 131700) ORF 129, (da 131790 a  
133283) ORF 130, (da 133246 a 133920) ORF 131, (da 133972 a 134370) ORF 132,

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(da 134418 a 134693) ORF 133a, (da 134402 a 134992) ORF R1, (da 134853 a 134419) ORF R2r, (da 135628 a 135897) ORF R3, (da 136780 a 137112) ORF R4, e (da 137558 a 137022) ORF R5r. Il gruppo di frammenti include gli omologhi dei frammenti esemplificativi elencati sopra, ossia frammenti omologhi di un altro PPVO (ad es. specie o ceppi come descritto sopra). Esso include anche varianti funzionali dei frammenti esemplificativi elencati sopra (la funzione essendo definita come per i frammenti in generale sopra). Dunque, il gruppo di frammenti include varianti con un'identità di sequenza di almeno l'80%, l'85%, il 90%, 95%, 96%, 97%, 98% o preferibilmente almeno il 99% con quei frammenti codificati dalle sequenze codificanti esemplificative di SEQ ID NO: 3 elencate sopra.

**[0018]** Il virione di PPVO inattivato può essere ottenuto con qualsiasi sistema noto nella tecnica adatto per inattivare i virus con involucro, per esempio mediante inattivazione fisica (ad es. esposizione al calore, specificamente pastorizzazione, o luce UV) o mediante inattivazione chimica (ad es. per mediante esposizione a pH basso, ad un detergente o ad un agente di inattivazione come l'etilenimmina, l'etilenimmina binaria, la formaldeide, la glutaraldeide, la 2,2'-ditiopiridina o il beta-propiolattone). In una forma di realizzazione preferita, il virione di PPVO inattivato è un virione inattivato con etilenimmina o etilenimmina binaria, preferibilmente un virione inattivato con etilenimmina binaria.

**[0019]** L'agente PPVO può comprendere delle informazioni genetiche (DNA o RNA) codificanti per un antigene eterologo (ossia non di parapoxvirus), e/o un immunomodulatore, o in alternativa può non comprendere informazioni genetiche codificanti per un antigene eterologo e/o un immunomodulatore. In una forma di

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realizzazione, esso non comprende informazioni genetiche codificanti per un antigene eterologo, ma facoltativamente comprende informazioni genetiche codificanti per un immunomodulatore.

**[0020]** In una forma di realizzazione preferita, l'agente PPVO è il PPVO di tipo selvatico. Preferibilmente è un virione di PPVO, più preferibilmente un virione di PPVO inattivato (iPPVO).

*Modalità d'uso*

**[0021]** L'agente PPVO si può usare da solo o in combinazione con un ulteriore regime di trattamento per (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto.

**[0022]** In una forma di realizzazione preferita, sia del preparare sia del trattare, l'ulteriore regime di trattamento può comprendere il somministrare un agente coronavirus.

**[0023]** Specificamente per il preparare, l'ulteriore regime di trattamento può comprendere il somministrare un ulteriore agente stimolatore immunitario (ad es. un adiuvante, un agonista di TLR, un PRR, ad es. un PRR citosolico come i recettori RIG-I-simili inclusi RIG-I, MDA5 e LGP2, o un mitogeno, particolarmente un mitogeno o un attivatore delle cellule T come la fitoemagglutinina (PHA), la Concanavalina (Con) A, l'agglutinina di germe di grano (WGA), il mitogeno della fitolacca (PWM), una lectina o un anticorpo anti-CD3 o un frammento di legame all'antigene di esso), oppure un mitogeno o un attivatore delle cellule B come PWM, il lipopolisaccaride (LPS), un anticorpo anti-CD40 o un frammento di legame all'antigene di esso, o un anticorpo anti-(recettore dell'antigene delle cellule B) o un frammento di legame all'antigene di esso. L'agente stimolante immunitario

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preferibilmente promuove uno stato cellulare antivirale. Specificamente per il trattare, l'ulteriore regime di trattamento può comprendere il somministrare uno o più ulteriori medicinali e/o una o più ulteriori terapie (trattamenti non medicamentosi/farmacologici) adatte per il trattamento di un soggetto infettato da un coronavirus o avente una malattia da coronavirus. Gli uno o più ulteriori medicinali sono preferibilmente selezionati dal gruppo costituito da un ulteriore agente stimolatore immunitario (come esemplificato sopra), un antivirale, un antibiotico, un adiuvante, un glucocorticoide, un farmaco anti-ipertensivo (ad es. un ACE inibitore), un farmaco ipoglicemico, un farmaco anti-shock e un farmaco adatto per trattare uno o più sintomi di una malattia da coronavirus e/o per accompagnare le una o più ulteriori terapie (ad es. un attenuatore del dolore, un sedativo, un farmaco anti-febbre, un farmaco anti-infiammatorio come l'IL-10, un farmaco per la tosse o un immunosoppressore). Le una o più ulteriori terapie sono preferibilmente selezionate dal gruppo costituito da una terapia con ossigeno e un supporto d'organo extra-corporeo (ECOS). La terapia con ossigeno può essere passiva (ossia che si basa sulla respirazione del soggetto) o attiva (ossia che fornisce ossigeno ai polmoni senza basarsi sulla respirazione del soggetto). Le terapie passive sono la terapia con ossigeno non pressurizzato e la terapia con ossigeno pressurizzato. Le terapie attive sono la ventilazione meccanica e l'ossigenazione extra-corporea a membrana (ECMO, anche una forma specifica di ECOS). Esempi di ECOS includono l'ECMO, la terapia sostitutiva renale continua (CRRT), l'emofiltrazione (HF) e l'emoperfusione (HP), ad es. per eliminare le citochine infiammatorie, e l'assistenza ventricolare sinistra.

**[0024]** Esempi specifici di ulteriori medicinali per preparare e trattare sono: Ivermectina,

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inibitori di TMPRSS2 (ad es. cloruro di ammonio, l'inibitore di serin proteasi camostat mesilato) facoltativamente insieme a un inibitore di CatB/L (ad es. E-64d), Favipiravir, Pimodivir, Baloxavir facoltativamente insieme a Marboxil, bloccanti dell'IL-6 (ad es. sarilumbab, tocilizumab), Sarilumab, Zitivekumab, lopinavir facoltativamente insieme a ritonavir, cloroquina, idrossicloroquina, Remdesivir,  $\alpha$ -Ketoammidi (ad es. ciclopentilmetil, cicloesilmetil o  $\alpha$ -Ketoamide 13b), Talidomide, Maraviroc, Tenofovir facoltativamente insieme a Disoproxil, Dolutegravir, Nelfinavir, Raltegravir, Tipranavir ed interferoni di Tipo I (IFN-I, specificamente IFN- $\beta$ 1). Gli esempi preferiti sono Remdesivir e Talidomide.

**[0025]** L'agente PPVO può essere somministrato per qualsiasi via, compresa ma non limitata alla via endovenosa, intramuscolare, orale, parenterale, topica, intradermica o sottocutanea. In una forma di realizzazione specifica, può essere somministrato direttamente nel tratto respiratorio, preferibilmente nel tratto respiratorio inferiore, più preferibilmente nel polmone (veicolazione polmonare). Come tale, può essere somministrato come aerosol e/o per inalazione.

**[0026]** La dose dell'agente PPVO (in effetti di tutti gli agenti descritti in questo testo, inclusi un ulteriore agente stimolatore immunitario, un ulteriore medicamento ed un agente coronavirus) è una dose farmaceuticamente efficace come può essere determinata dall'esperto. Per esempio, nel caso in cui l'agente sia un virione (vivo o inattivato), essa può essere nell'intervallo da  $10^6$  a  $10^{10}$  particelle. La dose si può somministrare una volta o più di una volta, per esempio per un periodo di < 12 settimane, < 6 settimane, < 4 settimane, < 2 settimane, o < 1 settimana.

*Coronavirus*

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**[0027]** Il coronavirus è, in una forma di realizzazione preferita, un alfa-coronavirus o un beta-coronavirus. Più preferibilmente è un coronavirus respiratorio, ossia un coronavirus che provoca una malattia respiratoria. Esempi di alfa-coronavirus sono HCoV-229E e CoV-NL63, ed esempi di beta-coronavirus sono SARS-CoV, SARS-CoV-2, MERS-CoV, HCoV-HKU1 e HCoV-OC43. È preferito che il coronavirus sia un coronavirus MERS o SARS, in cui preferibilmente il coronavirus SARS è un virus della specie "*coronavirus correlato a sindrome respiratoria acuta grave*", come classificato dal Comitato Internazionale sulla Tassonomia dei Virus (*International Committee on Taxonomy of Viruses*, ICTV). Nella forma di realizzazione maggiormente preferita, esso è SARS-CoV-2.

**[0028]** L'esperto rileva che i coronavirus mutano e che nuovi ceppi di SARS-CoV-2 sono isolati giornalmente, con 461 ceppi che sono stati isolati fino al 6 aprile 2020. Dunque, in termini di una definizione di sequenza, un virus preferito dell'invenzione ha un genoma comprendente o costituito da una sequenza secondo SEQ ID NO: 1 (ceppo originario del focolaio 2019 "Coronavirus da sindrome respiratoria acuta grave 2 isolato Wuhan-Hu-1", Sequenza di Riferimento in NCBI NC\_045512, versione del 30 marzo 2020) o una variante di essa con un'identità di sequenza di almeno l'80%, 85%, il 90%, 95%, 96%, 97%, 98% o preferibilmente almeno il 99%. In alternativa o in aggiunta, un virus preferito dell'invenzione ha un genoma comprendente o costituito da una sequenza secondo SEQ ID NO: 2 (una sequenza consenso di SARS-CoV-2 di 153 assemblaggi genomici) o una variante di essa con un'identità di sequenza di almeno l'80%, 85%, il 90%, 95%, 96%, 97%, 98% o preferibilmente almeno il 99%. È preferito che la variante sia classificata come un ceppo di SARS-CoV-2 dal Comitato Internazionale sulla Tassonomia dei Virus

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(*International Committee on Taxonomy of Viruses, ICTV*).

*Agente coronavirus*

**[0029]** Un agente coronavirus in generale è un agente che è adatto per trattare un'infezione da coronavirus. Può essere (a) il coronavirus stesso o un frammento di esso che è riconosciuto da e preferibilmente stimola una risposta immunitaria (ad es. un antigene e preferibilmente immunogeno), (b) un agente che comprende delle informazioni genetiche codificanti per (a), o (c) un agente che interferisce con l'infezione di una cellula da parte del coronavirus o con la replicazione del coronavirus all'interno di una cellula (in un contesto terapeutico: è in grado in seguito alla somministrazione di una quantità efficace di esso a un soggetto infettato con un coronavirus d'interferire con l'infezione di una cellula da parte del coronavirus o con la replicazione del coronavirus all'interno di una cellula).

L'agente coronavirus è preferibilmente scelto dal gruppo costituito da

- (i) un coronavirus inattivato,
- (ii) un frammento antigenico, preferibilmente immunogenico di un coronavirus (ad es. la proteina *spike* o un frammento di essa),
- (iii) un acido nucleico codificante per (i) o (ii),
- (iv) un vettore comprendente l'acido nucleico di (iii),
- (v) una cellula comprendente l'acido nucleico di (iii) o il vettore di (iv),
- (vi) un anticorpo o un frammento di legame all'antigene di esso che si lega a un coronavirus o ad un frammento di esso, preferibilmente l'involucro,
- (vii) un analogo nucleotidico/nucleosidico, preferibilmente specifico per un coronavirus,

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- (viii) una molecola di RNAi specifica per un coronavirus,
- (ix) una piccola molecola, un anticorpo o un frammento di legame all'antigene di esso, che si lega a un sito d'interazione proteina-proteina (PPI) tra una proteina di un coronavirus e una proteina del soggetto, e
- (x) plasma ematico di un soggetto che ha recuperato da un'infezione da coronavirus, preferibilmente da una malattia da coronavirus.

**[0030]** Un coronavirus può essere inattivato come descritto sopra per PPVO.

**[0031]** La PPI di (ix) è preferibilmente un'interazione di una proteina di SARS-CoV-2 o un omologo di essa di un altro coronavirus e una proteina del soggetto (l'ID della proteina Uniprot di ciascuna proteina del soggetto è < nome indicato di seguito >\_ HUMAN) selezionata da

- SARS-CoV2 E e AP3B1, BRD4, BRD2, CWC27, ZCH18 o CTL2;
- SARS-CoV2 M e MPPB, YIF1A, AT1B1, ACADM, ETFA, STOM, VKGC, VATA, PSMD8, REEP5, MPPA, ANO6, PREP, ZNT9, FAKD5, ZNT7, GCP3, COQ8B, SAAL1, REEP6, INT4, ODC, GCP2, SYTM, RTN4, FA8A1, AASS, AKP8L, AAR2 o BZW2
- SARS-CoV2 N e U3IP2, PABP1, CSK22, CSK2B, G3BP1, PABP4, LARP1, FA98A, SNIP1, RENT1, MOV10, G3BP2, DDX21, RBM28 o RL36;
- SARS-CoV2 Spike e ACE2, GOGA7 o ZDHC5;
- SARS-CoV2 nsp1 e DPOLA, PRI1, PRI2, DPOA2, GT251 o PKP2;
- SARS-CoV2 nsp10 e AP2A2, ALR, ERG11, AP2M1 o GRPE1;
- SARS-CoV2 nsp11 e TBCA;

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- SARS-CoV2 nsp12 e SBNO1, BCKD, AKAP8, MYCB2, SLU7, RIPK1, UBP2L, TYSD1, PDZ11, PRC2B, UBAP2, ZN318, CRT3, UBP54, Z3H7A, LAR4B, RBM41, HTF4, PPIL3 o PKHA5;
- SARS-CoV2 nsp13 e TBKB1, CTRO, HSBP1, PCNT, FR1OP, KAP2, KAPCA, KAP3, RADI, CENPF, TLE1, TLE3, TLE5, GOGA3, GOGA2, GGOB1, GRAP1, CE350, MYOME, CP135, CEP68, CNTRL, RB6I2, GCC2, CLIP4, NIN, CE112, MIPO1, UBP13, GCC1, JKIP1, CK5P2, AKAP9, GORS1, FYCO1, CA050, CP250, TBK1, HOOK1 o NINL;
- SARS-CoV2 nsp14 e AGAL, IMDH2 o SIR5;
- SARS-CoV2 nsp15 NTF2, ARF6 o RNF41;
- SARS-CoV2 nsp2 e S27A2, IF4E2, NCPR, GDS1, WASC4, FKB15 o GGYF2;
- SARS-CoV2 nsp4 e IDE, TIM10, ALG11, PO210, TIM29, DJC11, T10B o TIM9;
- SARS-CoV2 nsp5 e HDAC2;
- SARS-CoV2 nsp5\_C145A e GPX1 o TRM1;
- SARS-CoV2 nsp6 e ATP5L, VAS1, SGMR1 o AT133;
- SARS-CoV2 nsp7 e ADAS, CYB5B, ACSL3, NB5R3, RALA, COMT, RAB5C, RAB7A, RAB8A, RAB2A, RAB10, RAB14, RHOA, RAB1A, GBB1, GBG5, LMAN2, MOGS, TOIP1, MARC1, QSOX2, HS2ST, NDUF2, SCPDL, SCRBI, NAT14, DCAKD, F162A, TIM14, SELS, PGES2 o RAB18;
- SARS-CoV2 nsp8 e MPP10, SRP72, ATE1, NSD2, SRP19, SRP54, RT25, DDX10, LARP7, MEPCE, NGDN, EXOS8, SYNM, NOL10, CCD86, SPCS, EXOS5, EXOS3, AATF, HECD1, RT02, RT05, EXOS2 o RT27;

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- SARS-CoV2 nsp9 e T2FB, FBN1, FBN2, NU214, NUP62, DCAF7, IF4H, NUP54, MIB1, NEK9, ZN503, NUP88, NUP58, MAT2B o FBLN5;
- SARS-CoV2 orf10 e PPT1, CUL2, MA7D1, THTPA, ZY11B, TIM8B, RBX1, ELOC o ELOB;
- SARS-CoV2 orf3a e HMOX1, TRI59, AR6P6, VPS39, CLCC1, VPS11, SUN2 o ALG5;
- SARS-CoV2 orf3b e STML2;
- SARS-CoV2 orf6 e NUP98, RAE1L o MTCH1;
- SARS-CoV2 orf7a e HEAT3 o MDN1;
- SARS-CoV2 orf8 e PLOD2, TOR1A, STC2, TPA, ITB1, CISD3, CO6A1, PVR, DNMT1, LYOX, PCSK6, INHBE, NPC2, MFGM, OS9, NPTX1, PLGT2, PLGT3, EROIB, PLD3, FXRD2, CHSS2, PUSL1, EMC1, GGH, ERLEC, I17RA, NGLY1, H6ST2, SDF2, NEUR1, GDF15, TM2D3, ERP44, EDEM3, SIL1, OFUT1, SMOC1, PKHF2, FXL12, UGGG2, CHPF2, ATS1, HYOU1, FKBP7, ADAM9 o FKB10;
- SARS-CoV2 orf9b e NHRF1, CHM2A, CSDE1, TOM70, MARK3, MARK2, DPH5, DCTP1, MARK1, PTBP2 o BAG5; e
- SARS-CoV2 orf9c e UBXN8, GPAA1, WFS1, MRP1, PAR2, SCAP, D19L1, SGMR2, ZNT6, TAPT1, ERMP1, NLRX1, RETR3, PIGO, FACR2, ECSIT, ALG8, TM39B, GHITM, ACAD9, NFIP2, BCS1, CIA30, TMED5, NDUB9 o PIGS.

**[0032]** Un anticorpo o un frammento di legame all'antigene di esso per un sito di PPI può essere generato con metodi di routine. Per esempio, anticorpi o loro frammenti di legame all'antigene per una delle proteine interagenti possono essere generati e quindi vagliati per

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interferire con l'interazione.

**[0033]** La piccola molecola di (ix) è preferibilmente selezionata dal gruppo costituito da Loratadina, Daunorubicina, Midostaurina, Ponatinib, Silmitasertib, Acido Valproico, Aloperidolo, Metformina, Migalastat, S-verapamil, Indometacina, Ruxolitinib, Acido micofenolico, Entacapone, Ribavirina, E-52862, Merimepodib, RVX-208, XL413, AC-55541, Apicidina, AZ3451, AZ8838, Bafilomicina A1, CCT 365623, GB110, H-89, JQ1, PB28, PD-144418, RS-PPCC, TMCB, UCPH-101, ZINC1775962367 ZINC4326719 e ZINC4511851, ZINC95559591, 4E2RCat, ABBV-744, Camostat, Captopril, CB5083, Cloramfenicolo, Cloroquina, Composto 10 inibitore di PI4K-III $\beta$  (Rutaganira, F. U. et al., J. Med. Chem. 59, 1830-1839 (2016)), Composto 2 Inibitore della Ciclofillina (Mackman, R. L. et al., J. Med. Chem. 61, 9473-9499 (2018)), CPI-0610, Dabrafenib, DBeQ, dBET6, IHVR-19029, Linezolid, Lisinopril, Minoxidil, ML240, MZ1, Nafamostat, Pevonedistat, PS3061, Rapamicina (Sirolimus), Sangliferina A, Sapanisertib (INK128/M1N128), FK-506 (Tacrolimus), Ternatina 4 (DA3), Tigeciclina, Tomivosertib (eFT-508), Verdinexor, WD002, Azacitidina, Selinexor, e Zotatifina (eFT226).

#### *Infezione/malattia da Coronavirus*

**[0034]** Un'infezione da coronavirus è definita dall'ingresso del coronavirus in almeno una cellula di un soggetto e la sua replicazione nell'almeno una cellula. Preferibilmente, l'infezione è un'infezione del tratto respiratorio, che include il tratto respiratorio superiore (naso e passaggi nasali, seni paranasali, la faringe e la porzione della laringe al di sopra delle pieghe (corde) vocali) e il tratto respiratorio inferiore (porzione della laringe al di sotto delle pieghe vocali, trachea, bronchi, bronchioli e i polmoni, che includono i

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bronchioli respiratori, i dotti alveolari, le sacche alveolari e gli alveoli). Più preferibilmente, è un'infezione del tratto respiratorio inferiore, con preferenza massima dei polmoni (inclusi uno o più di bronchioli respiratori, dotti alveolari, sacche alveolari e alveoli). L'infezione può inoltre essere caratterizzata immunologicamente dalla presenza di almeno un fattore immunitario specifico per antigeni di coronavirus, preferibilmente selezionato dal gruppo costituito da cellule B, cellule T helper follicolari (cellule T<sub>FH</sub>), cellule T CD4<sup>+</sup> attivate e cellule T CD8<sup>+</sup> (in particolare anche CD38<sup>+</sup>HLA-DR<sup>+</sup>), anticorpi IgM e anticorpi IgG. In alternativa o in aggiunta, può essere caratterizzata immunologicamente da un profilo di citochine specifico per coronavirus, preferibilmente nel sangue o nel sito d'infezione come specificato sopra (con preferenza massima nel polmone). Questi profili possono essere determinati dall'esperto senza indebito carico, ad es. prelevando un campione di tessuto, ad es. dal polmone, infettandolo con il coronavirus e determinando l'espressione di citochine. Esempi di profili di citochine specifici per i coronavirus sono (a) per SARS-CoV: regolazione positiva di uno o più, preferibilmente tutti di IFN-I (ad es. IFN-β), IFN-II (IFN-γ), IFN-III (IFN-λ1, IFN-λ2 e IFN-λ3), IL-1beta, IL-6, IL-12, IL-8, MCP-1, MIP-1alfa, RANTES, CXCL1, CXCL2, CXCL5, e CXCL9, e preferibilmente nessuna regolazione positiva di CXCL10; e (b) per SARS-CoV-2: regolazione positiva di uno o più, preferibilmente tutti di IL-6, MCP-1, CXCL1, CXCL5, e/o CXCL10, e preferibilmente nessuna regolazione positiva di qualsiasi IFN e/o di uno qualsiasi o tutti di IL-1beta, IL-12, IL-8, MIP-1alfa, RANTES, CXCL2 e CXCL9.

**[0035]** Il soggetto preferito è l'uomo. Mentre al meglio della conoscenza degli inventori il coronavirus infetta tutti gli esseri umani in modo uguale, ci sono dei fattori di rischio di

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contrarre un'infezione da coronavirus:

- (i) Recente (entro il periodo d'incubazione, ossia nei precedenti 27, 24, o 19 giorni, preferibilmente 14 (CDC, NHC) o 10 (OMS) giorni) presenza o viaggio da un'area con diffusione in corso di infezione da coronavirus. L'area è preferibilmente una "Area Interessata", determinata dall'OMS. "Recente" può significare entro il periodo di incubazione del coronavirus, preferibilmente entro gli ultimi 27, 24, o preferibilmente 19 giorni, più preferibilmente 14 giorni o con preferenza massima 10 giorni.
- (ii) Contatto con una persona infettata con coronavirus, in particolare una persona avente uno o più sintomi di una malattia da coronavirus. "Contatto" può significare esposto a goccioline respiratorie della persona (ad es. nello spazio delle goccioline respiratorie nell'aria o in contatto con superfici toccate dalla persona o dalle goccioline respiratorie).
- (iii) Essere in una professione a rischio elevato, che solitamente richiede di essere a contatto con il pubblico generale (il contatto essendo definito come sopra) regolarmente (ad es. almeno giornalmente o almeno settimanalmente), ad es. essere un medico professionista, in particolare in un ospedale, essere un operatore sanitario (ad es. per bambini o anziani) o un operatore al dettaglio (per esempio in un supermercato o una farmacia).

**[0036]** Un'infezione da coronavirus può causare o non causare i sintomi di una malattia da coronavirus in un soggetto. I termini "infezione da coronavirus" e "malattia da coronavirus" si distinguono qui per la presenza di almeno un sintomo di una malattia da coronavirus. Fintanto che l'infezione non è accompagnata da almeno un sintomo di una malattia da coronavirus, essa (o il soggetto) è asintomatica (include presintomatica). Il termine

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"malattia da coronavirus" nell'uso fatto in questo testo richiede la presenza di un'infezione da coronavirus e almeno un sintomo di una malattia da coronavirus (qui indicata anche come "infezione sintomatica").

**[0037]** I sintomi del coronavirus includono tosse secca, febbre ( $\geq 37,8^{\circ}\text{C}$ ), naso che gocciola e/o bloccato, affaticamento, difficoltà respiratoria, polmonite, insufficienza d'organo (ad es. cuore, polmone, fegato e/o rene), prurito alla gola, mal di testa, dolore articolare, nausea, diarrea, brividi, linfopenia, perdita dell'olfatto e/o perdita del gusto. Preferibilmente, la malattia da coronavirus è caratterizzata dalla presenza di due o più, tre o più o quattro o più sintomi, preferibilmente inclusi uno o due o più tra tosse secca, febbre ( $\geq 37,8^{\circ}\text{C}$ ), difficoltà respiratoria, perdita dell'olfatto e/o perdita del gusto.

**[0038]** La malattia da coronavirus è preferibilmente una malattia respiratoria (ad es. SARS o MERS), più preferibilmente Covid-19. La malattia da coronavirus può assumere un decorso lieve (non grave) o grave. Un decorso lieve è caratterizzato dalla presenza di uno o più sintomi soltanto leggeri (ossia nessun sintomo grave) di una malattia da coronavirus. I sintomi lievi sono selezionati dal gruppo costituito da tosse secca, febbre lieve (da  $\geq 37,8^{\circ}\text{C}$  a  $< 40^{\circ}\text{C}$ ), naso che gocciola e/o bloccato, affaticamento, prurito alla gola, mal di testa, dolore articolare, nausea, diarrea, brividi, linfopenia, perdita dell'olfatto e perdita del gusto. I sintomi gravi sono selezionati dal gruppo costituito da difficoltà respiratoria, in particolare sindrome da insufficienza respiratoria acuta, polmonite, insufficienza d'organo (ad es. cuore, polmone, fegato e/o rene) e febbre alta ( $\geq 40^{\circ}\text{C}$ ). Un decorso grave è caratterizzato clinicamente da uno o più di (i) la presenza di uno o più sintomi gravi di una malattia da coronavirus, (ii) necessità di ospedalizzazione, in particolare necessità di

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ricovero in unità di terapia intensiva (ICU) e/o necessità di una terapia con ossigeno (come definita sopra), in particolare di un respiratore (definito anche ventilatore), e/o (iii) morte del soggetto. Una necessità di ospedalizzazione è caratterizzata da una o più tra: evidenze cliniche o radiologiche di polmonite, sindrome da insufficienza respiratoria acuta e/o la presenza di due o più sintomi che includono almeno febbre e tosse persistente (con o senza espettorato), in particolare tosse persistente (ad es. di almeno 3 giorni). Una necessità di ricovero in ICU è caratterizzata da polmonite grave e/o una o più complicazioni gravi. La polmonite grave è caratterizzata da tachipnea (età inferiore a 2 mesi: 60 o più respiri al minuto; età da 2 a 11 mesi: 50 o più respiri al minuto, età da 1 a 5 anni: 40 o più respiri al minuto, età > 5 anni: 30 o più respiri al minuto), insufficienza respiratoria e/o ossigenazione inadeguata (ad es., SpO<sub>2</sub> del 93% o meno per gli adulti e 90% o meno per i bambini). Le complicanze gravi sono complicanze che sono letali quando non trattate e includono, per esempio, shock settico, sindrome da insufficienza respiratoria acuta e insufficienza d'organo. In alternativa o in aggiunta, un decorso grave può essere caratterizzato immunologicamente ad es. dalla presenza o dallo sviluppo di una tempesta di citochine, in particolare nel polmone. Una tempesta di citochine da coronavirus è caratterizzata da iperinflammazione, preferibilmente da un aumento (rispetto al soggetto infetto ma asintomatico, preferibilmente come dopo il periodo di incubazione, o al valore intermedio o la media di una pluralità di tali soggetti) di uno o più tra IL-2, IL-7, TNF-alfa, MIP-1alfa, MCP-1, G-CSF e/o CXCL10, in cui l'aumento può essere caratterizzato come eccessivo e/o incontrollato. In particolare, la tempesta di citochine è una tempesta di citochine da Covid-19. Inoltre, la tempesta di citochine è preferibilmente nel tratto respiratorio come

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definito per l'infezione di cui sopra (incluse le parti preferite di esso).

**[0039]** Mentre la malattia da coronavirus può prendere un decorso grave in qualsiasi soggetto indipendentemente dall'età o dalla condizione pre-esistente, ci sono tuttavia dei fattori di rischio di avere un decorso grave di una malattia da coronavirus, tra cui:

- (i) età, ad es. almeno 40, 50, 60, 70, o 80 anni con il rischio che aumenta con l'età,
- (ii) genere maschile,
- (iii) fumo, e
- (iv) avere una o più comorbidità, preferibilmente selezionate dal gruppo costituito da malattia cardiovascolare, diabete, malattia respiratoria (in particolare cronica), ipertensione, AIDS, cancro, malattia epatica, malattia renale e malattia polmonare. Una comorbidità particolare come fattore di rischio è una malattia respiratoria.

*Preparare un soggetto*

**[0040]** La preparazione di un soggetto è intesa per preparare un soggetto per un'infezione da coronavirus, che si ottiene con l'immunomodulazione risultante nell'uso medico dell'invenzione. Può essere denominata anche “trattamento preparatorio”, “trattamento profilattico” oppure, rispetto alla malattia risultante dall'invenzione, “trattamento preventivo” o “trattamento preventivo”. Non s'intende impedire necessariamente un'infezione da coronavirus. Come tale, il soggetto non è ancora infettato dal coronavirus.

**[0041]** Dunque, il preparare può avere uno o più effetti immunologici o (preferibilmente e) può avere uno o più effetti clinici, entrambi una volta che un soggetto è infettato. Questi effetti immunologici sono selezionati preferibilmente dal gruppo costituito da:

- (I-i) aumentare il numero di uno o più dei fattori immunitari specifici per antigeni di

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coronavirus (come sopra definiti) nel soggetto o in una parte del soggetto,

(I-ii) inibire (ossia ridurre o arrestare) la replicazione di coronavirus nel soggetto o in una parte del soggetto,

(I-iii) ridurre il carico di un coronavirus nel soggetto o in una parte del soggetto, e

(I-iv) prevenire o migliorare un profilo di citochine o una tempesta di citochine specifica per coronavirus (come definito sopra) nel soggetto o in una parte del soggetto.

Qui, la parte del soggetto è preferibilmente il tratto respiratorio (tratto respiratorio superiore o preferibilmente inferiore, più preferibilmente il polmone). (I-ii) è preferito rispetto a (I-i), e (I-iii) rispetto a (I-ii). Inoltre (I-ii) può comportare (I-i) e (I-iii) può comportare (I-i) e/o (I-ii). (I-iv) è generalmente desiderato.

**[0042]** Gli effetti clinici sono preferibilmente selezionati dal gruppo costituito da:

(C-i) ridurre la contagiosità,

(C-ii) ritardare l'insorgenza di una malattia da coronavirus,

(C-iii) ridurre il grado di uno o più sintomi prospettici di una malattia da coronavirus, preferibilmente di uno o più sintomi prospettici gravi di una malattia da coronavirus,

(C-iv) prevenire uno o più sintomi di una malattia di coronavirus, preferibilmente uno o più sintomi gravi di una malattia di coronavirus,

(C-v) prevenire un decorso grave di una malattia da coronavirus, e

(C-vi) prevenire una malattia da coronavirus.

Qui, (C-ii) è preferito rispetto a (C-i), (C-iii) rispetto a (C-ii), (C-iv) rispetto a (C-iii), (C-v) rispetto a (C-iv) e (C-vi) rispetto a (C-v).

**[0043]** I sintomi prospettici sono sintomi che non sono ancora presenti ma possono

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presentarsi o si presenteranno. Dunque, la riduzione del grado dei sintomi prospettici si riferisce ad un trattamento profilattico dei sintomi.

**[0044]** Di conseguenza, l'agente PPVO può essere per l'uso in uno o più da (I-i) a (I-iv) e/o uno o più da (C-i) a (C-vi) in un soggetto non ancora infettato da un coronavirus. In altre parole, l'agente PPVO è in grado, in seguito alla somministrazione di una quantità efficace di esso, di raggiungere uno o più da (I-i) a (I-iv) e/o uno o più da (C-i) a (C-vi).

**[0045]** In una forma di realizzazione preferita, il soggetto da preparare è caratterizzato da uno o più fattori di rischio di contrarre un'infezione da coronavirus e/o da uno o più fattori di rischio di avere un decorso grave di una malattia da coronavirus.

**[0046]** In un'altra forma di realizzazione, il soggetto da preparare è selezionato con un metodo secondo il corrispondente ulteriore aspetto dell'invenzione descritto di seguito.

*Trattare un soggetto*

**[0047]** Il trattamento di un'infezione da coronavirus (ossia di un soggetto che è infetto) non è inteso necessariamente per trattare o prevenire una malattia da coronavirus, sebbene sia preferito che lo faccia. Come tale, il trattamento può essere di un'infezione asintomatica (ossia di un soggetto infetto non avente sintomi) o di una malattia da coronavirus (ossia di un soggetto infetto avente uno o più sintomi).

**[0048]** Il trattamento può avere uno o più effetti immunologici o (preferibilmente e) può avere uno o più effetti clinici. Questi effetti immunologici sono preferibilmente come definiti per la preparazione di cui sopra, o per un'infezione asintomatica o per la malattia. Gli effetti clinici per un'infezione asintomatica sono preferibilmente selezionati dal gruppo costituito da (C-i) a (C-vi) come definito per la preparazione di cui sopra. Gli effetti clinici

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per un'infezione sintomatica (ossia su una malattia da coronavirus) sono preferibilmente selezionati dal gruppo costituito da

(C-i), (C-vi), entrambi come definiti per il preparare di cui sopra,

(C-vii) migliorare uno o più (o idealmente tutti) sintomi di una malattia da coronavirus,

(C-viii) prevenire, uno o più (o idealmente tutti) gli ulteriori sintomi di una malattia da coronavirus, e

(C-ix) migliorare, preferibilmente prevenire, un decorso grave di una malattia da coronavirus.

Qui, gli “ulteriori sintomi” sono quelli che non caratterizzano ancora l’infezione sintomatica.

**[0049]** Di conseguenza, l'agente PPVO può essere per l'uso in uno o più da (I-i) a (I-iv) e/o uno o più da (C-i) a (C-i) in un soggetto infettato da coronavirus. In altre parole, l'agente PPVO è in grado, con la somministrazione di una quantità efficace di esso, di ottenere uno o più da (I-i) a (I-iv) e/o uno o più da (C-i) a (C-ix). In una forma di realizzazione preferita, l'agente PPVO è per l'uso nel prevenire o trattare una malattia da coronavirus.

**[0050]** Come indicato sopra, il soggetto da trattare può essere asintomatico. In alternativa, il soggetto da trattare può avere una malattia da coronavirus. Il soggetto avente una malattia da coronavirus può non avere dei sintomi gravi, ad es. ha soltanto uno o più sintomi blandi. In un'altra forma di realizzazione, può avere un decorso grave di una malattia da coronavirus (eccetto (iii) la morte; preferibilmente (i) uno o più sintomi gravi). In una forma di realizzazione preferita, il soggetto da trattare (ad es. uno qualsiasi dei soggetti sopra menzionati eccetto quelli che hanno già un decorso grave di una malattia da

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coronavirus) è caratterizzato da uno o più fattori di rischio di avere un decorso grave di una malattia da coronavirus.

**[0051]** In un'ulteriore forma di realizzazione preferita, il soggetto è caratterizzato da almeno uno tra:

- (i) assenza di tempesta di citochine nel polmone, e preferibilmente assenza completa di tempesta di citochine,
- (ii) età fino a 75, 70 o preferibilmente 65 anni, più preferibilmente da 18 a 65 anni, e/o
- (iii) HScore di 169 o minore, preferibilmente 150 o minore, 130 o minore, o 110 o minore.

Preferibilmente, il soggetto è caratterizzato da (ii) e/o (iii), (ii) e HScore di 169 o minore, o (iii) ed età da 18 a 65 anni.

**[0052]** L'HScore genera una probabilità per la presenza di linfoistocitosi emofagocitica secondaria (sHLH), una sindrome iperinflammatoria con un profilo di tempesta di citochine simile a quello di una malattia del coronavirus (Pehta et al., The Lancet Vol 395 March 28, 2020). Uno HScore maggiore di 169 è al 93% sensibile e all'86% specifico per la SHLH. L'HScore si calcola usando i seguenti criteri: temperatura (<38,4°C: 0 punti, 38,4-39,4°C: 33 punti, >39,4°C: 49 punti), organomegalia (assenza: 0 punti, epatomegalia o splenomegalia: 23 punti, epatomegalia e splenomegalia: 38 punti), numero di citopenie\* (un lignaggio: 0 punti, due lignaggi: 24 punti, tre lignaggi: 34 punti), trigliceridi (<1,5 mmoli/l: 0 punti, 1,5-4,0 mmoli/l: 44 punti, > 4,0 mmoli/l: 64 punti), fibrinogeno (>2,5 g/L: 0 punti, ≤2,5 g/L: 30 punti), ferritina (<2000 ng/ml: 0 punti, 2000-60000 ng/ml: 35 punti, >60000 ng/ml: 50 punti), aspartato amminotrasferasi nel siero (<30 UI/L: 0 punti, ≥30

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IU/L: 19 punti), emofagocitosi su aspirato di midollo osseo (no: 0 punti, sì: 35 punti), e immunosoppressione nota† (no: 0 punti, sì: 18 punti). \*Definita come concentrazione di emoglobina di 9,2 g/dL o minore ( $\leq 5,71$  mmoli/L), una conta di globuli bianchi di 5000 globuli bianchi per  $\text{mm}^3$  o minore, o una conta piastrinica di 110000 piastrine per  $\text{mm}^3$  o minore, o tutti questi criteri combinati. †positivo a HIV o che riceve una terapia immunosoppressiva a lungo termine (ad es. uno o più di glucocorticoidi, ciclosporina e/o azatioprina).

**[0053]** In un'altra forma di realizzazione, il soggetto da trattare è selezionato con un metodo secondo il corrispondente ulteriore aspetto dell'invenzione descritto di seguito.

*Ulteriore uso medico*

**[0054]** In un ulteriore aspetto di uso medico, l'invenzione si riferisce a un agente PPVO per l'uso come un medicamento che promuove uno stato cellulare antivirale (preferibilmente anticoronavirale) in un soggetto (i) avente un'infezione da coronavirus o (ii) caratterizzato da uno o più fattori di rischio di contrarre un'infezione da coronavirus e/o da uno o più fattori di rischio di avere un decorso grave di malattia da coronavirus.

**[0055]** In una forma di realizzazione, gli uno o più fattori di rischio di contrarre un'infezione da coronavirus sono selezionati dai fattori (i) e (ii) come definito sopra.

**[0056]** In un'altra forma di realizzazione, gli uno o più fattori di rischio di avere un decorso grave di una malattia da coronavirus comprendono almeno (ii) e (iii), o (ii) e (iv) come definiti sopra, o comprendono almeno una comorbidity selezionata tra malattia respiratoria (in particolare cronica) e malattia polmonare. In aggiunta, l'età del soggetto è preferibilmente almeno 60, almeno 70, o più preferibilmente almeno 80 anni.

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**[0057]** La frase "promuovere uno stato cellulare antivirale" si riferisce alla promozione della trascrizione di geni antivirali cellulari codificanti per proteine della difesa dell'ospite, ad es. come promosso da IFN- $\alpha$  e/o  $\beta$ . Preferibilmente inibisce (ossia previene o almeno riduce) la replicazione virale nella cellula e/o induce apoptosi (per prevenire l'ulteriore replicazione virale nella cellula), e più preferibilmente riduce la quantità di virioni infettivi rilasciati da una cellula infettata rispetto a una cellula in cui lo stato cellulare antivirale non è stato promosso dal medicamento. In questo modo, si arresta o almeno si rallenta la propagazione del virus. "Promuovere" include un'induzione (ad es. per le cellule non ancora in uno stato antivirale) e un rafforzamento (ossia potenziamento) (ad es. per le cellule già in uno stato antivirale). Anche se non limitata a questa, la prima è preferita per un soggetto che non ha (ancora) un'infezione da coronavirus, ad es. caratterizzato da uno o più fattori di rischio come specificato sopra, e il secondo è preferito per un soggetto avente un'infezione da coronavirus.

#### *Metodi di trattamento*

**[0058]** Sono inoltre esposti i seguenti metodi, che sono da interpretare come riferimenti agli agenti e alle composizioni dell'invenzione per l'uso in un metodo per preparare un soggetto per un'infezione o trattare un'infezione:

Un metodo per somministrare un agente PPVO ad un soggetto (i) avente un'infezione da coronavirus o (ii) caratterizzato da uno o più fattori di rischio di contrarre un'infezione da coronavirus e/o da uno o più fattori di rischio di avere un decorso grave di malattia da coronavirus.

**[0059]** Un metodo per promuovere uno stato cellulare antivirale (preferibilmente

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anticoronavirale) in un soggetto (i) avente un'infezione da coronavirus o (ii) caratterizzato da uno o più fattori di rischio di contrarre un'infezione da coronavirus e/o da uno o più fattori di rischio di avere un decorso grave di malattia da coronavirus, il metodo comprendendo il somministrare un agente PPVO al soggetto.

**[0060]** Un metodo per (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto, il metodo comprendendo somministrare un agente PPVO al soggetto.

**[0061]** Un metodo per trattare una malattia da coronavirus in un soggetto, il metodo comprendendo somministrare un agente PPVO al soggetto.

**[0062]** Le definizioni e le forme di realizzazione descritte di seguito, in particolare con il titolo "Definizioni e ulteriori forme di realizzazione dell'invenzione" si applicano al primo aspetto dell'invenzione.

**[0063]** In un secondo aspetto, l'invenzione si riferisce a una composizione o un kit comprendente un agente PPVO e un agente coronavirus.

**[0064]** In una forma di realizzazione preferita, la composizione o il kit comprende inoltre uno o più diluenti, veicoli e/o conservanti farmaceuticamente accettabili; e/o è una composizione farmaceutica o un kit. I diluenti, veicoli e/o conservanti farmaceuticamente accettabili sono descritti di seguito e possono in aggiunta a questi includere un mezzo (ad es. acquoso) adatto a mantenere strutturalmente un parapoxvirus, in particolare un parapoxvirus inattivato, o un liofilizzato di questo mezzo. Specificamente, la composizione o il kit possono essere per qualsiasi degli usi medici dell'invenzione descritta in questo testo.

**[0065]** La composizione può essere formulata per qualsiasi via di somministrazione come

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definita sopra. Inoltre, essa può essere formulata per la somministrazione simultanea o separata o per il rilascio simultaneo o separato dell'agente PPVO e dell'agente coronavirus.

**[0066]** In una forma di realizzazione preferita, il kit comprende una composizione del secondo aspetto.

**[0067]** In un'altra forma di realizzazione, il kit comprende un foglietto con le istruzioni per l'uso dell'agente PPVO e dell'agente coronavirus, o della composizione del secondo aspetto, preferibilmente secondo un uso medico qui descritto.

**[0068]** Le definizioni date e le forme di realizzazione descritte in riferimento al primo aspetto si applicano anche al secondo aspetto, nella misura in cui sono applicabili. Inoltre, le definizioni e le forme di realizzazione descritte di seguito, in particolare con il titolo "Definizioni e ulteriori forme di realizzazione dell'invenzione" si applicano alla composizione o al kit del secondo aspetto.

**[0069]** In un terzo aspetto, l'invenzione si riferisce a una composizione farmaceutica o un kit per qualsiasi degli usi medici dell'invenzione descritta in questo testo, la composizione farmaceutica o il kit comprendendo un agente PPVO e preferibilmente un ulteriore medicamento. La composizione farmaceutica solitamente comprende uno o più diluenti, veicoli e/o conservanti farmaceuticamente accettabili.

**[0070]** In una forma di realizzazione preferita, il kit comprende una composizione del terzo aspetto.

**[0071]** In un'altra forma di realizzazione, il kit comprende un foglietto con le istruzioni per l'uso dell'agente PPVO e dell'ulteriore medicamento, o della composizione del terzo aspetto, preferibilmente secondo un uso medico qui descritto.

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**[0072]** Le definizioni date e le forme di realizzazione descritte in riferimento al primo e al secondo aspetto (ad es. la formulazione) applicano anche al terzo aspetto, nella misura in cui sono applicabili. Inoltre, le definizioni e le forme di realizzazione descritte di seguito, in particolare con il titolo "Definizioni e ulteriori forme di realizzazione dell'invenzione" si applicano alla composizione o al kit del terzo aspetto.

**[0073]** In un ulteriore aspetto, l'invenzione riguarda un metodo per selezionare un soggetto per qualsiasi degli usi medici dell'invenzione descritta in questo testo, il metodo comprendendo i passaggi di (i) determinare la presenza o assenza di una tempesta di citochine nel soggetto, e (ii) selezionare il soggetto se una tempesta di citochine è assente.

**[0074]** In una forma di realizzazione, il passaggio (i) comprende determinare l'HScore del soggetto, e il passaggio (ii) comprende selezionare il soggetto se l'HScore è 169 o minore.

**[0075]** In un'altra forma di realizzazione, il passaggio (i) comprende rilevare in un campione biologico del soggetto uno o più segni d'iperinfiammazione, e il passaggio (ii) comprende selezionare il soggetto se non è rilevabile alcun segno d'iperinfiammazione. Iperinfiammazione in questo contesto è un livello elevato, ad es. eccessivo e/o incontrollato, (rispetto a un soggetto infetto ma asintomatico, preferibilmente come dopo il periodo di incubazione, o il valore intermedio o la media di una pluralità di tali soggetti) di uno o più tra IL-2, IL-7, TNF-alfa, MIP-1alfa, MCP-1, G-CSF e/o CXCL10; o di uno o più tra IL-6, ferritina, conta piastrinica e/o velocità di sedimentazione degli eritrociti (ESR). Il campione biologico è preferibilmente selezionato dal gruppo costituito da sangue o un campione derivato dal sangue (ad es. plasma o siero), espettorato, saliva, e un campione derivato dal polmone (ad es. per broncoscopia inclusi il lavaggio bronchiale, il lavaggio alveolare

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bronchiale, lo spazzolamento bronchiale e l'abrasione bronchiale).

**[0076]** Il metodo del quarto aspetto è preferibilmente un metodo *ex vivo*.

**[0077]** Le definizioni date e le forme di realizzazione descritte in riferimento al primo, secondo e terzo aspetto si applicano anche al quarto aspetto, nella misura in cui sono applicabili.

Inoltre, le definizioni e le forme di realizzazione descritte di seguito, in particolare con il titolo "Definizioni e ulteriori forme di realizzazione dell'invenzione" si applicano al metodo del quarto aspetto.

#### Definizioni e ulteriori forme di realizzazione dell'invenzione

**[0078]** La specifica usa una serie di termini e frasi che hanno certi significati come definiti sotto. I significati preferiti sono da interpretare come forme di realizzazione preferite degli aspetti dell'invenzione descritti in questo testo. Come tali, queste ed anche ulteriori forme di realizzazione descritte nel seguito possono essere combinate con qualsiasi forma di realizzazione degli aspetti dell'invenzione e in particolare qualsiasi forma di realizzazione preferita degli aspetti dell'invenzione descritti sopra.

**[0079]** Il termine "identità" o "identica" nel contesto delle sequenze polinucleotidiche si riferisce al numero di residui nelle due sequenze che sono identici quando allineati per la massima corrispondenza. Specificamente, l'identità di sequenza percentuale di due sequenze è il numero di corrispondenze esatte tra due sequenze allineate diviso per la lunghezza della sequenza più corta e moltiplicato per 100. Gli strumenti di allineamento che si possono usare per allineare due sequenze sono ben noti all'esperto nella tecnica e si possono per esempio ottenere sul World Wide Web, ad es. Needle (EMBOSS)

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([https://www.ebi.ac.uk/Tools/psa/emboss\\_needle/](https://www.ebi.ac.uk/Tools/psa/emboss_needle/)), MUSCLE  
(<http://www.ebi.ac.uk/Tools/msa/muscle/>), MAFFT (<http://www.ebi.ac.uk/Tools/msa/mafft/>) o WATER ([http://www.ebi.ac.uk/Tools/psa/emboss\\_water/](http://www.ebi.ac.uk/Tools/psa/emboss_water/)). Gli allineamenti tra due sequenze si possono eseguire usando le impostazioni predefinite dei parametri, ad es. per Needle preferibilmente MATRIX: BLOSUM62, Gap Open: 10.0, Gap Extend: 0.5, per MAFFT preferibilmente: Matrix: Blosum62, Gap Open 1.53, Gap Extend 0.123, per i polinucleotidi in WATER preferibilmente: MATRIX: DNAFULL, Gap Open: 10.0, Gap Extend 0.5 e per i polipeptidi in WATER preferibilmente: MATRIX: BLOSUM62, Gap Open: 10.0, Gap Extend: 0.5. Gli esperti del ramo comprendono che può essere necessario introdurre degli spazi in una delle due sequenze per produrre un allineamento soddisfacente. Il "miglior allineamento di sequenza" è definito come l'allineamento che produce il maggior numero di residui identici allineati avendo un numero minimo di spazi. Preferibilmente, è un allineamento globale che include ogni residuo in ogni sequenza nell'allineamento.

**[0080]** Il termine "variante" si riferisce in generale ad una versione modificata del polinucleotide, ad es. una mutazione, per cui uno o più nucleotidi del polinucleotide possono essere mutati. Generalmente, la variante è funzionale, il che significa ad es. in riferimento a un virus che il virus è in grado di infettare una cellula ospite. La funzionalità è generalmente quella descritta per il polinucleotide da cui deriva la variante. Una "mutazione" può essere una sostituzione, delezione e/o inserzione nucleotidica ("e" può essere pertinente se c'è più di una mutazione). Preferibilmente si tratta di una sostituzione.

**[0081]** Il termine "antigene" si riferisce a qualsiasi sostanza, ad es. una proteina o un

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peptide, che è in grado di essere legato da un anticorpo, un recettore delle cellule B e/o un recettore delle cellule T. Un antigene comprende almeno un epitopo preferibilmente comprendente almeno 8 amminoacidi e più preferibilmente comprendente tra 8 e 17 amminoacidi. L'epitopo può essere un epitopo delle cellule T e/o delle cellule B. Un epitopo delle cellule T è un epitopo che può essere presentato sulla superficie di una cellula presentante l'antigene, dove è legato ad una molecola del MHC. Nell'uomo, le cellule professionali che presentano l'antigene sono specializzate per presentare i peptidi del MHC di classe II, mentre la maggior parte delle cellule somatiche nucleate presentano i peptidi del MHC di classe I. Gli epitopi delle cellule T presentati da molecole del MHC di classe I sono tipicamente dei peptidi di lunghezza tra 8 e 11 amminoacidi, mentre le molecole del MHC di classe II presentano dei peptidi più lunghi, ad es. 13-17 amminoacidi di lunghezza. Un epitopo delle cellule B è un epitopo che è riconosciuto come strutture tridimensionali sulla superficie di antigeni nativi dalle cellule B. Gli epitopi possono essere previsti con strumenti *in silico*, ad es. gli strumenti online di previsione delle cellule B o T della IEDB Analysis Resource.

**[0082]** Il termine "immunogeno" si riferisce ad un antigene che è in grado di indurre una risposta immunitaria.

**[0083]** "Recettore di riconoscimento dei profili" o "PRR", nell'uso fatto in questo testo, si riferisce a un sensore dell'ospite codificato dalla linea germinale che rileva un profilo molecolare associato a patogeni (PAMP), che è specifico per un patogeno, e/o un profilo molecolare associato a danni (DAMP), che è specifico per i componenti di una cellula ospite che sono rilasciati durante il danno o la morte della cellula. Un PRR può essere un

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PRR legato alla membrana (PRR della superficie cellulare) o un PRR citosolico.

**[0084]** Un “adiuvante” è una sostanza che accelera, prolunga e/o potenzia la qualità e/o la forza di una risposta immunitaria ad un antigene/immunogeno, in confronto alla somministrazione del solo antigene, dunque riducendo la quantità di antigene/immunogeno necessaria, e/o la frequenza di iniezione necessaria al fine di generare una risposta immunitaria adeguata all’antigene/immunogeno d’interesse. Esempi di adiuvanti che si possono usare nel contesto della composizione secondo la presente invenzione sono precipitati simili a gel di idrossido di alluminio (allume);  $AlPO_4$ ; alidrogel; prodotti batterici della membrana esterna di batteri Gram-negativi, in particolare il monofosforil lipide A (MPLA), lipopolisaccaridi (LPS), muuramil dipeptidi e derivati di questi; adiuvante incompleto di Freund; liposomi, in particolare liposomi neutri, liposomi contenenti la composizione ed eventualmente citochine; copolimeri a blocchi non ionici; adiuvante ISCOMATRIX (Drane et al., 2007); DNA non metilato comprendente dinucleotidi CpG (motivo CpG), in particolare CpG ODN con uno scheletro di fosforotioato (PTO) (CpG PTO ODN) o uno scheletro di fosfodiesterio (PO) (CpG PO ODN); derivati lipopeptidici sintetici, in particolare Pam<sub>3</sub>Cys; lipoarabinomannano; peptidoglicano; zimosano; proteine dello shock termico (HSP), in particolare HSP 70; dsRNA e derivati sintetici di esso, in particolare Poly I:C; peptidi policationici, in particolare poli-L-arginina; tassolo; fibronectina; flagellina; imidazochinolina; citochine con attività adiuvante, in particolare GM-CSF, interleuchina (IL-)2, IL-6, IL-7, IL-18, interferoni di tipo I e II, in particolare IFN- $\gamma$ , TNF- $\alpha$ ; 25-diidrossivitamina D3 (calcitriolo); e oligopeptidi sintetici, in particolare peptidi presentati dal MHC-II. I polimeri a blocchi

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non ionici contenenti poliossietilene (POE) e poliossipropilene (POP), come i copolimeri a blocchi POE-POP-POE, si possono usare come adiuvante (Newman et al., 1998). Questo tipo di adiuvante è particolarmente utile per le composizioni comprendenti acidi nucleici come ingrediente attivo.

**[0085]** Il termine “ricombinante” si riferisce in particolare ad un virus che è modificato per comprendere una sequenza polinucleotidica eterologa nel suo genoma.

**[0086]** Il termine "immunomodulatore" si riferisce a un farmaco usato per regolare o normalizzare il sistema immunitario inducendo, esaltando, sopprimendo e/o indebolendo una risposta immunitaria in un soggetto ("e" significando che alcune parti del sistema immunitario sono selettivamente indotte o potenziate e altre sono selettivamente soppresse o indebolite). Esempi sono citochine, agonisti o antagonisti non citochine dei recettori delle citochine (ad es. anticorpi o piccoli composti), anticorpi o piccoli composti che legano (preferibilmente neutralizzano) citochine, e recettori solubili delle citochine (ad es. per intrappolare le citochine). Non include un antigene.

**[0087]** Il termine "vettore" nell'uso fatto in questo testo comprende qualsiasi vettore noto all'esperto, compresi i vettori plasmidici, vettori cosmidici, vettori fagici come il fago lambda, vettori virali come i vettori adenovirus (Ad), vettori virus adeno-associati (AAV), vettori alfavirus (ad es. il virus dell'encefalite equina Venezuelana (VEE), virus sindbis (SIN), virus della foresta semliki (SFV), e chimere VEE-SIN), vettori herpes virus, vettori virus del morbillo, vettori virus del vaiolo (ad es., virus vaccinia, il virus vaccinia modificato Ankara (MVA), NYVAC (derivato dal ceppo Copenaghen della vaccinia), e vettori del vaiolo aviario: vaiolo dei canarini (ALVAC) e vaiolo dei polli (FPV), e vettori

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virus della stomatite vescicolare, o particelle simili a virus. Nell'uso fatto in questo testo, il termine "particella simile a virus" o "VLP" si riferisce a un guscio virale vuoto non replicante. Le VLP sono generalmente composte da una o più proteine virali quali, ma non limitate a quelle denominate proteine del capsido, del rivestimento, del guscio, di superficie e/o dell'involucro. Esse contengono proteine virali funzionali responsabili della penetrazione cellulare da parte del virus che garantisce un ingresso efficiente nella cellula. I metodi per produrre delle VLP particolari sono noti nella tecnica.

**[0088]** Il termine "mitogeno" si riferisce ad un agente, ad es. un peptide o una proteina che induce una cellula ad iniziare la divisione cellulare. Il termine "attivatore cellulare" si riferisce ad un agente, ad es. un peptide o una proteina che si lega e forma legami crociati con i recettori cellulari, ad es. i recettori delle cellule T o delle cellule B.

**[0089]** Il termine "analogo nucleosidico" si riferisce a un nucleoside contenente un analogo di un acido nucleico e uno zucchero. Il termine "analogo nucleotidico" si riferisce a un nucleotide contenente un analogo di acido nucleico, uno zucchero e un gruppo fosfato con 1-3 fosfati. Il termine "analogo di un acido nucleico" si riferisce ad un composto che è strutturalmente simile all'RNA e al DNA in quanto ha una o più tra catena principale di fosfati, uno zucchero pentoso e/o la base nucleotidica alterata in modo tale da avere proprietà diverse di accoppiamento delle basi e/o di impilamento delle basi. Gli esempi includono acido nucleico peptidico (PNA), acido nucleico morfolino e bloccato (LNA), nonché acido nucleico glicol (GNA) e acido nucleico treosio (TNA).

**[0090]** Il termine "molecola di RNAi" si riferisce a una molecola in grado di neutralizzare le molecole di mRNA in una maniera specifica per la sequenza bersaglio. Gli esempi

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includono dsRNA, miRNA e siRNA.

**[0091]** Le forme farmaceutiche particolarmente preferite per la somministrazione degli agenti secondo l'invenzione sono forme adatte per uso iniettabile e includono soluzioni o dispersioni acquose sterili e polveri sterili per la preparazione estemporanea di soluzioni o dispersioni iniettabili sterili. Tipicamente, una tale soluzione o dispersione includerà un solvente o mezzo di dispersione, contenente, per esempio, soluzioni acquose in tampone acquoso, ad es. tamponi biocompatibili, etanolo, un poliolo come il glicerolo, propilene glicol, polietilene glicol, miscele opportune di questi, tensioattivi od oli vegetali. Le soluzioni per infusione o iniezione possono essere realizzate con un numero qualsiasi di tecniche riconosciute nell'arte che includono, ma non sono limitate all'aggiunta di conservanti come agenti anti-batterici o anti-fungini, ad es. paraben, clorobutanolo, fenolo, acido sorbico o tiomersale. Inoltre, agenti isotonici, come zuccheri o sali, in particolare cloruro di sodio, possono essere incorporati nelle soluzioni per infusione o iniezione.

**[0092]** I diluenti preferiti della presente invenzione sono acqua, tamponi fisiologici accettabili, soluzioni saline tampone fisiologicamente accettabili o soluzioni saline. Gli eccipienti che si possono usare con le varie forme farmaceutiche del farmaceutico secondo l'invenzione possono essere scelti dal seguente elenco non limitativo:

- a) leganti quali lattosio, mannitolo, sorbitolo cristallino, fosfati dibasici, fosfati di calcio, zuccheri, cellulosa microcristallina, carbossimetil cellulosa, idrossietil cellulosa, polivinil pirrolidone e simili,
- b) lubrificanti quali magnesio stearato, talco, calcio stearato, zinco stearato, acido stearico, olio vegetale idrogenato, leucina, gliceridi e sodio stearyl fumarati,

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- c) disintegranti quali amidi, croscarmellosio, sodio metil cellulosa, agar, bentonite, acido alginico, carbossimetil cellulosa, polivinil pirrolidone e simili.

**[0093]** Il termine "veicolo farmaceuticamente accettabile" si riferisce a qualsiasi substrato che serve a migliorare la selettività, l'efficacia e/o la sicurezza della somministrazione di farmaci. Tali veicoli possono essere usati per controllare il rilascio di un farmaco nella circolazione sistemica. Questo può essere realizzato o mediante lento rilascio del farmaco per un lungo periodo di tempo (tipicamente diffusione) o mediante rilascio indotto sul bersaglio del farmaco da qualche stimolo, come variazioni di pH, applicazione di calore, e attivazione mediante la luce. I veicoli si possono anche usare per migliorare le proprietà farmacocinetiche, specificamente la biodisponibilità, di molti farmaci con scarsa solubilità in acqua e/o permeabilità di membrana. È stata sviluppata e studiata un'ampia varietà di sistemi di veicoli di farmaci. Gli esempi includono liposomi, micelle polimeriche, microsfele, nanoparticelle, nanofibre, coniugati proteina-farmaco, eritrociti, virosomi e dendrimeri. Si possono usare diversi metodi per attaccare il farmaco al veicolo, inclusi adsorbimento, integrazione nella struttura in massa, incapsulamento, e legame covalente.

**[0094]** L'invenzione è descritta a titolo dei seguenti esempi che devono essere intesi come puramente illustrativi e non limitativi dell'ambito dell'invenzione.

ESEMPIO 1

#### Obiettivo

**[0095]** Testare l'attività antivirale di virioni inattivati di parapoxvirus *orf* (iPPVO) contro il coronavirus. I virioni PPVO stimolano il braccio innato e anche adattativo del sistema

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immunitario.

#### Approccio

**[0096]** Nel sangue intero, le APC (cellule che presentano l'antigene) captano il virione iPPVO, il che porta alla loro attivazione e al rilascio di citochine. Questo a sua volta attiva le cellule T e anche le cellule NK che pure cominciano a proliferare e a rilasciare citochine. Dopo 3 giorni d'incubazione le cellule sono centrifugate e si raccoglie il supernatante contenente fattori solubili come le citochine. Questi supernatanti sono saggiati per la loro attività su coronavirus.

#### Sostanze in esame

##### **[0097]**

- iPPVO del ceppo D1701 (disponibile da Pfizer); 230 unità di IFN ricostituite in 2 ml di acqua
- veicolo di iPPVO (solo tampone PPVO, ossia senza virus), controllo negativo
- PBS, controllo negativo
- Concavalina (Con) A, co-stimolo, soluzione madre in PBS 1 mg/ml = 10X (usata nel pozzetto a 5 µg/ml)

#### Ulteriori materiali

##### **[0098]**

- Sangue intero di un donatore sano: 9 ml di sangue intero raccolto fresco in una Monovette Sarstedt, Li-Eparina
- Terreno per l'incubazione del sangue intero: RPMI (500 ml) integrato con GlutaMAX™ all'1% e 0,25 ml di Eparina-Sodio (5000 U).

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- Cellule del saggio del coronavirus: VeroE6-EGFP propagate in un terreno di crescita preparato integrando il DMEM con FCS inattivato al calore al 10% v/v e 5 mL di bicarbonato di sodio al 7,5%, e coltivate in fiasca T150 e divise 1/4 due volte a settimana. Pen-strep aggiunti direttamente nella fiasca T150 a una diluizione 1/100.
- Terreno per il saggio del coronavirus: preparato integrando il DMEM con FCS inattivato al calore al 2% v/v di e 5 mL di bicarbonato di sodio al 7,5%.

#### Preparazione dei campioni

**[0099]** Per pozzetto in una piastra a fondo tondo da 96 pozzetti: 120 µl di terreno delle cellule per l'incubazione del sangue intero + 20 µl di ConA o PBS + 20 µl di iPPVO, veicolo di iPPVO o PBS + 40 µl di sangue intero.

**[0100]** La piastra è stata incubata a 37 °C, 5% di CO<sub>2</sub> per 72 h e quindi centrifugata per 5 min a 230 g. Il supernatante è stato aliquotato in nuove piastre da 96 pozzetti (disposizione della piastra mantenuta). Le piastre dei campioni sono state congelate a -80 °C fino a ulteriore uso.

#### Saggio del Coronavirus

**[0101]** Diluizione dei campioni (sostanza in esame preparata come sopra): 100 µL di terreno del saggio sono stati aggiunti ai pozzetti di una piastra Greiner Bio One 655090 e 15 µL sono stati prelevati nuovamente dai pozzetti (tranne che dai pozzetti per i controlli di questo saggio: il controllo del virus (VC) che controllava per la replicazione del virus e l'effetto sulle cellule, e il controllo delle cellule (CC) per controllare la vitalità cellulare senza virus). 15 µL dei pozzetti della piastra dei campioni sono stati aggiunti nei pozzetti, di cui sono stati prelevati 15 µL di terreno.

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**[0102]** Preparazione della sospensione cellulare: Una coltura confluyente (monostrato) di VeroE6-EGFP in fiasca T150 è stata lavata con DPBS e sono stati aggiunti 10 mL di tripsina 0,25% tripsina/EDTA. La fiasca è stata incubata per 1 minuto a temperatura ambiente, e la tripsina/EDTA è stata eliminata eccetto 2 mL. La fiasca è stata quindi incubata per 15 minuti a 37°C. Dopo l'incubazione, le cellule sono state risospese in 10 mL di terreno del saggio, fatte passare attraverso un Cell Strainer (FALCON n° di Cat 352350) e contate usando il coulter (sono stati contati 3 campioni di 10 µL in 10 mL). 3000 cellule sono state quindi risospese in 50 µL di terreno del saggio e 50 µL della sospensione cellulare sono stati seminati in ciascun pozzetto della piastra Greiner.

**[0103]** Saggio: Le piastre con la sospensione cellulare preparate come sopra sono state incubate per una notte (37°C / 5% di CO<sub>2</sub>), dopodiché SARS-CoV-2 (ceppo BetaCoV/Belgio/GHB-03021/2020, titolo della soluzione madre 4,8 x 10<sup>7</sup> TCID<sub>50</sub>/ml) è stato aggiunto in tutti i pozzetti (eccetto i pozzetti del controllo cellulare) ad una diluizione finale di 1/200.0000 in 200 µl/pozzetto (3000 cellule/pozzetto, dando come risultato una MOI di 0,016 TCID<sub>50</sub>/cellula). Le piastre sono state incubate a 37°C / 5% di CO<sub>2</sub> per 5 giorni, dopodiché il segnale della GFP è stato misurato usando la fluorescenza nel pozzetto intero, HCl (*High Content Imaging*, visualizzazione ad alto contenuto) e il software ImageJ. Una ridotta espressione di EGFP è un marcatore dell'attività citopatica indotta dal virus. Vedi anche (Journal of Virological Methods 129, 2005, 56-63).

### Risultati

**[0104]** I risultati sono mostrati in Fig. 1. Le cellule non infettate con il coronavirus (CC media) erano vitali ed esprimevano eGFP. Le cellule infettate con il coronavirus (VC

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media) morivano e non era rilevabile un'espressione di eGFP. I risultati per PBS e il veicolo di iPPVO da solo erano simili alla VC media, vale a dire PBS e il veicolo di iPPVO da solo non hanno avuto un effetto sulla vitalità cellulare, ossia contro il coronavirus, come previsto. Anche ConA 5 µg/ml da sola (in PBS) non ha mantenuto l'espressione di eGFP, e non ha neppure influenzato l'espressione di eGFP quando è stata data insieme al veicolo di iPPVO come mostrato dal confronto con la ConA da sola in PBS. Anche iPPVO D1701 da solo (in PBS) ha avuto un chiaro effetto positivo sulla vitalità cellulare, e l'espressione di eGFP è stata ulteriormente aumentata quando iPPVO D1701 è stato usato in combinazione con 5 µg/ml di ConA (che di per sé non ha mostrato un effetto sull'espressione di eGFP a questa concentrazione). Di conseguenza, iPPVO e ConA agiscono sinergicamente.

**[0105]** Questi risultati mostrano l'efficacia del parapoxvirus nel promuovere uno stato cellulare antivirale che è protettivo e inibisce la replicazione e la propagazione del coronavirus e quindi l'infezione di ulteriori cellule. Questo effetto può essere migliorato aggiungendo un agente stimolatore immunitario come ConA che agisce sinergicamente, ossia l'efficacia di iPPVO è potenziata in un approccio combinatorio.

## ESEMPIO 2

### Obiettivo

**[0106]** Determinare se la prova di principio mostrata nell'Esempio 1 è applicabile al parapoxvirus in generale testando l'attività antivirale di un ulteriore ceppo di parapoxvirus contro il coronavirus.

### Approccio

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**[0107]** Si veda l'Esempio 1.

Sostanze in esame

**[0108]**

- iPPVO del ceppo NZ2 (inattivato chimicamente con etilenimmina binaria, BEI), denominazione interna AIC649,  $1 \times 10^9$  virioni liofilizzati ricostituiti in 1 ml di acqua
- iPPVO del ceppo D1701, vedi Esempio 1
- Veicolo di iPPVO, controllo negativo, vedi Esempio 1

Ulteriori materiali, preparazione dei campioni, saggio del coronavirus

**[0109]** Si veda l'Esempio 1. iPPVO e il veicolo di iPPVO sono stati usati da soli. iPPVO D1701 che ha dimostrato di aumentare la vitalità cellulare nell'Esempio 1 è stato usato come controllo positivo nell'Esempio 2.

Risultati

**[0110]** I risultati sono mostrati in Fig. 2. iPPVO D1701, che nell'Esempio 1 si è dimostrato efficace, è stato usato come controllo positivo e come parametro di riferimento del 100%. Entrambi i ceppi di PPVO D1701 e NZ2 sono attivi contro il coronavirus come dimostrato dal confronto con il controllo negativo. Di conseguenza, la promozione di uno stato cellulare antivirale che è efficace contro il coronavirus è un segno distintivo del parapoxvirus in generale e non solo del ceppo testato nell'Esempio 1.

ESEMPIO 3

Obiettivo

**[0111]** Indagare la tempistica dell'aggiunta del parapoxvirus, specificamente determinare come l'attività antivirale del parapoxvirus dimostrata negli Esempi 1 e 2 è modulata

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aggiungendo i supernatanti in diversi punti temporali alle cellule del saggio del coronavirus.

Approccio

**[0112]** Si vedano gli Esempi 1 e 2, ma con diversi punti temporali per l'aggiunta dei supernatanti della piastra dei campioni alle cellule del saggio del coronavirus (prima o dopo l'infezione delle cellule VeroE6-EGFP con SARS-CoV-2).

Sostanze in esame

**[0113]**

- iPPVO del ceppo NZ2, vedi Esempio 2
- iPPVO del ceppo D1701, vedi Esempio 1
- Veicolo di iPPVO, controllo negativo, vedi Esempio 1
- ConA, vedi Esempio 1

Ulteriori materiali, preparazione dei campioni, saggio del coronavirus

**[0114]** Si vedano gli Esempi 1 e 2. Il sangue intero è stato incubato con iPPVO o il veicolo di iPPVO (da solo o in combinazione con ConA). I supernatanti sono stati generati e aggiunti a diversi punti temporali prima o dopo l'infezione delle cellule VeroE6-EGFP con il coronavirus (SARS-CoV-2).

ESEMPIO 4

Obiettivo

**[0115]** Testare ulteriori approcci combinatori, specificamente confermando che la forza dell'attività antivirale del parapoxvirus dimostrata negli Esempi 1, 2 e 3 può essere potenziata negli approcci combinatori usando ulteriori controparti di combinazione, ad es. un agente stimolante immunitario o un antivirale come descritto in questo testo. Questo

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include indagare la tempistica dell'aggiunta del parapoxvirus e delle controparti di combinazione (parapoxvirus nei campioni di sangue e controparte di combinazione nel campione di sangue o direttamente nelle cellule del saggio di coronavirus), ossia l'una rispetto all'altra, e anche la tempistica di aggiungere il coronavirus rispetto al parapoxvirus e alla controparte di combinazione. Questo include anche testare l'influenza delle concentrazioni di parapoxvirus e controparte di combinazione.

#### Approccio

**[0116]** Si vedano gli Esempi 1, 2 e 3, ma con diversi punti temporali per aggiungere i supernatanti (del sangue trattato con parapoxvirus e/o controparte di combinazione, allo stesso o a diversi punti temporali) della piastra dei campioni alle cellule del saggio del coronavirus (prima o dopo l'infezione delle cellule VeroE6-EGFP con SARS-CoV-2), e con diversi punti temporali per aggiungere la controparte di combinazione (se non aggiunta già nel campione di sangue) nelle cellule del saggio del coronavirus (prima o dopo l'aggiunta del sopranatante e/o l'aggiunta di SARS-CoV-2).

#### Sostanze in esame

##### **[0117]**

- iPPVO del ceppo NZ2, vedi Esempio 2
- iPPVO del ceppo D 1701, vedi Esempio 1
- Veicolo di iPPVO, controllo negativo, vedi Esempio 1
- ConA, vedi Esempio 1 (controllo della controparte di combinazione)
- Talidomide (controparte di combinazione)
- Remdesivir (controparte di combinazione)

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Ulteriori materiali, preparazione dei campioni, saggio del coronavirus

**[0118]** Si vedano gli Esempi 1 e 2. Il sangue intero è stato incubato con iPPVO o il veicolo di iPPVO (da solo o in combinazione con una o più controparti di combinazione). I supernatanti sono stati aggiunti a diversi punti temporali prima o dopo l'infezione delle cellule VeroE6-EGFP con il coronavirus (SARS-CoV-2). Una o più controparti di combinazione non usate per l'incubazione dei campioni di sangue sono state aggiunte a diversi punti temporali nelle cellule VeroE6-EGFP (prima sia del surnatante sia del coronavirus, dopo entrambi o tra i due).

Sono state usate diverse concentrazioni delle controparti di combinazione.

LISTA DELLE SEQUENZE

**[0119]**

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<110> AiCuris GmbH & Co.KG

<120> PARAPOXVIRUS FOR CONDITIONING FOR AND TREATMENT OF CORONAVIRUS INFECTIONS

<130> 1092-2 EP

<160> 3

<170> PatentIn version 3.5

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ga <u>uu</u> ugc <u>g</u> a gaa <u>u</u> g <u>c</u> ung cac <u>u</u> g <u>ca</u> ga aga <u>aa</u> cacg <u>c</u> aa <u>uu</u> aaugc cu <u>g</u> ucug <u>u</u> gu 4500
gg <u>aa</u> ac <u>ua</u> aa gcc <u>au</u> ag <u>uu</u> ca <u>ac</u> u <u>au</u> ca gcg <u>ua</u> aa <u>u</u> au aag <u>g</u> gu <u>uu</u> a aa <u>u</u> acaag <u>a</u> 4560
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### **RIVENDICAZIONI**

1. Un agente *Parapoxvirus ovis* (PPVO) per l'uso nel (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto.
2. L'agente PPVO secondo la rivendicazione 1, l'agente PPVO essendo:
  - (a) il PPVO stesso o un frammento di esso, o
  - (b) un agente che comprende delle informazioni genetiche codificanti per (a).
3. L'agente PPVO della rivendicazione 2, l'agente PPVO essendo un virione di PPVO inattivato.
4. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 3, in cui il coronavirus è un coronavirus SARS.
5. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 4, in cui il coronavirus è SARS-CoV-2 o una variante di esso con un'identità di sequenza di almeno l'80% con SEQ ID NO: 1 o 2.
6. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 5, in cui il soggetto da preparare è caratterizzato da uno o più fattori di rischio di contrarre un'infezione da coronavirus e/o da uno o più fattori di rischio di avere un decorso grave di una malattia da coronavirus.
7. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 5, in cui il soggetto da trattare è asintomatico.
8. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 5, in cui il soggetto da trattare ha una malattia da coronavirus, preferibilmente Covid-19.
9. L'agente PPVO per l'uso secondo la rivendicazione 8, in cui il soggetto non ha

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sintomi gravi oppure ha un decorso grave della malattia da coronavirus.

10. L'agente PPVO per l'uso secondo una qualsiasi delle rivendicazioni da 1 a 9, in cui l'uso comprende la somministrazione di un ulteriore regime di trattamento per (i) o (ii).

11. L'agente PPVO per l'uso secondo la rivendicazione 10, in cui l'ulteriore regime di trattamento per (i) comprende il somministrare un agente coronavirus e/o un ulteriore agente stimolante immunitario, e l'ulteriore regime di trattamento per (ii) comprende il somministrare un agente coronavirus, uno o più ulteriori medicinali e/o uno o più ulteriori trattamenti non farmacologici.

12. L'agente PPVO secondo la rivendicazione 11, in cui

- l'agente coronavirus è selezionato dal gruppo costituito da:
  - (i) un coronavirus inattivato,
  - (ii) un frammento antigenico, preferibilmente immunogenico, di un coronavirus,
  - (iii) un acido nucleico codificante per (i) o (ii),
  - (iv) un vettore comprendente l'acido nucleico di (iii),
  - (v) una cellula comprendente l'acido nucleico di (iii) o il vettore di (iv),
  - (vi) un anticorpo o un frammento di legame all'antigene di esso che si lega a un coronavirus o un frammento di esso, preferibilmente l'involucro,
  - (vii) un analogo nucleotidico/nucleosidico, preferibilmente specifico per un coronavirus,
  - (viii) una molecola di RNAi specifica per un coronavirus,
  - (ix) una piccola molecola, un anticorpo o un frammento di legame

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all'antigene di esso, che si lega a un sito d'interazione proteina-proteina (PPI) tra una proteina di coronavirus e una proteina del soggetto, e (x) plasma ematico di un soggetto che ha recuperato da un'infezione da coronavirus, preferibilmente da una malattia da coronavirus;

- l'ulteriore medicamento è selezionato dal gruppo costituito da un agente stimolante immunitario, un antivirale, un antibiotico, un adiuvante, un glucocorticoide, un farmaco anti-ipertensivo, un farmaco ipoglicemico, un farmaco anti-shock e un farmaco adatto per trattare uno o più sintomi di una malattia da coronavirus; e/o
- le una o più ulteriori terapie sono preferibilmente selezionate dal gruppo costituito da una terapia con ossigeno e un supporto d'organo extra-corporeo (ECOS).

13. L'agente PPVO per l'uso secondo la rivendicazione 12, in cui la piccola molecola è selezionata dal gruppo costituito da Loratadina, Daunorubicina, Midostaurina, Ponatinib, Silmitasertib, Acido Valproico, Aloperidolo, Metformina, Migalastat, S-verapamil, Indometacina, Ruxolitinib, Acido micofenolico, Entacapone, Ribavirina, E-52862, Merimepodib, RVX-208, XL413, AC-55541, Apicidina, AZ3451, AZ8838, Bafilomicina A1, CCT 365623, GB110, H-89, JQ1, PB28, PD-144418, RS-PPCC, TMCB, UCPH-101, ZINC1775962367, ZINC4326719, ZINC4511851, ZINC95559591, 4E2RCat, ABBV-744, Camostat, Captopril, CB5083, Cloramfenicolo, Cloroquina, Composto 10 inibitore di PI4K-III $\beta$ , Composto 2 inibitore della Ciclofillina, CPI-0610, Dabrafenib, DBeQ, dBET6, IHVR-19029, Linezolid, Lisinopril, Minoxidil, ML240, MZ1, Nafamostat, Pevonedistat,

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PS3061, Rapamicina (Sirolimus), Sangliferina A, Sapanisertib (INK128/M1N128), FK-506 (Tacrolimus), Ternatina 4 (DA3), Tigeciclina, Tomivosertib (eFT-508), Verdinexor, WDB002, Azacitidina, Selinexor, e Zotatifina (eFT226).

14. Una composizione o un kit comprendente un agente PPVO, preferibilmente come definito nella rivendicazione 2 o 3, e un agente coronavirus, preferibilmente come definito nella rivendicazione 12.

15. Una composizione farmaceutica o un kit per l'uso nel (i) preparare un soggetto per un'infezione da coronavirus o (ii) trattare un'infezione da coronavirus in un soggetto, la composizione farmaceutica o il kit comprendendo un agente PPVO, preferibilmente come definito nella rivendicazione 2 o 3, e preferibilmente un ulteriore medicamento, più preferibilmente come definito nella rivendicazione 12.

Dogana, 29/09/2022

In fede

  
Il Mandatario  
Ing. Marco Montebelli  
USBM CPI 001

B055.D1.SM.61E

**TRADUZIONE DELLE FIGURE**

**Figura 1, 2**

Figure

Figura

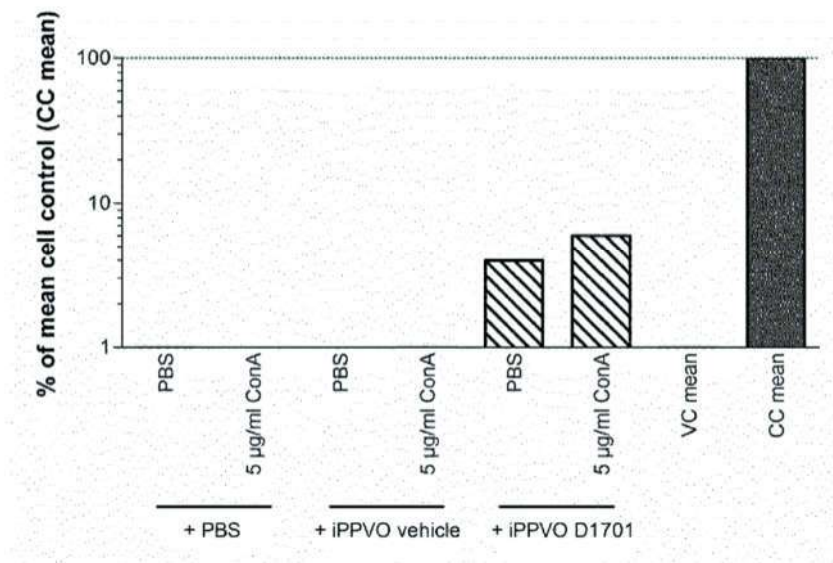
**Figura 1**

<b>% of mean cell control (CC mean)</b>	% del controllo cellulare medio (CC medio)
VC mean	VC medio
CC mean	CC medio
<b>+ iPPVO vehicle</b>	+ veicolo di iPPVO

**Figura 2**

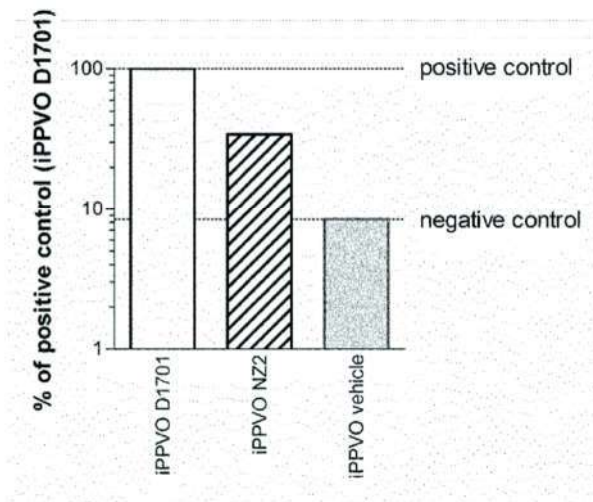
<b>% of positive control</b>	% del controllo positivo
<b>positive control</b>	controllo positivo
<b>negative control</b>	controllo negativo
<b>iPPVO vehicle</b>	veicolo di iPPVO

Figure 1



  
Ing. Marco Montebelli  
USBM CPI 001

Figure 2



  
Ing. Marco Montebelli  
USBM CPI 001