

Therapeutic antibodies with neutralizing activity against SARS-CoV-2 Glycoprotein S

Field of the Invention

The present invention is related to antibodies and antigen-binding fragments of antibodies that specifically bind the SARS-CoV-2 glycoprotein S (spike) as well as diagnostic and therapeutic methods of using those antibodies.

Background of the Invention

Coronavirus disease 2019 (COVID-19) is a highly infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Common symptoms of COVID-19 include fever, cough, fatigue, shortness of breath, and loss of smell and taste. While the majority of cases result in mild symptoms, some progress to acute respiratory distress syndrome (ARDS) characterized by a cytokine storm, multi-organ failure, septic shock, and blood clots. The time from exposure to onset of symptoms is typically around five days, but may range from two to fourteen days.

SARS-CoV-2 was previously referred to by its provisional name, "2019 novel coronavirus" (2019-nCoV). As described by the National Institutes of Health, it is the successor to "SARS-CoV-1" (or just "SARS-CoV"). It is believed to have zoonotic origins and has close genetic similarity to bat coronaviruses, suggesting it emerged from a bat-borne virus.

Taxonomically, SARS-CoV-2 belongs to the *coronaviridae*, positive-sense single-stranded RNA viruses. Coronaviruses are large, roughly spherical, particles with bulbous surface projections. The average diameter of the virus particles is around 125 nm (0.125 μ m). The diameter of the envelope is 85 nm and the spikes are 20 nm long.

The viral envelope consists of a lipid bilayer, in which the membrane (M), envelope (E) and spike (S) structural proteins are anchored. The ratio of E:S:M in the lipid bilayer is approximately 1:20:300 (wt/wt). On average a coronavirus particle has 74 surface spikes.

The coronavirus surface spikes are homotrimers of the glycoprotein protein S, which is composed of an S1 and S2 subunit.

It is believed that, similar to SARS-CoV and MERS, the virus mainly enters human cells by binding specifically to the receptor angiotensin converting enzyme 2 (ACE2). It is believed that the S1-domain of the spike glycoprotein binds host's ACE2-receptor and the S2 domain mediates

the viral fusion with the host cell membrane.

Although some similarities between SARS-CoV and SARS-CoV-2 exist, it has been found that SARS-CoV-2 S protein binds specifically ACE2 with significant higher affinity than SARS-CoV (10- to 20-fold). Furthermore, it was shown that antibody cross-reactivity is limited between the two virus S proteins, suggesting its recognition to ACE2 is different as compared to SARS-CoV.

Several published SARS-CoV Abs do not have appreciable binding specifically to SARS-CoV-2 S protein. A recent study (Tian et al., 2020) shows a SARS-CoV antibody, CR3022, which binds specifically to SARS-CoV-2 RBD (receptor binding domain), but to a highly conserved region outside of the ACE2-RBD-interaction site and with weak or absent neutralization capability.

As of today, globally more than 5.600.000 people are reported to be infected with SARS-CoV-2, and about 350.000 people died because of COVID-19.

Neutralizing antibodies of therapeutic quality, which prevent the virus from entering the host cell would be of vital importance as primary treatment, especially in the case of COVID-19 where no medication or vaccination has been identified.

Thus, a great need exists to develop neutralizing antibodies, which prevent the infection with SARS-CoV-2 in a quality suitable for therapeutic use.

Furthermore, it has been reported that SARS-CoV-2 shows "escape"-mutations. These are mutations especially in the RBD-region, which still enable the virus to bind to cellular ACE-2 protein via its spike-protein, but seem to allow the evasion of host's immune response.

This finding is of great importance for estimating the chances of a re-infection with SARS-CoV-2 after having successfully overcome a first SARS-CoV-2-infection, as well as having an impact on the development of vaccines and the treatment with antiviral agents, such as monoclonal antibodies.

Prior art attempts to overcome the danger of the virus building a resistance against certain treatments include the use of polyclonal antibodies or cocktails of two or more monoclonal antibodies, which bind to different epitopes of the target protein.

However, these strategies comprise other negative consequences. For example, polyclonal antibodies do show a wide range of specificity and, consequently, are difficult to predict in their efficacy. Therapeutics based on polyclonal antibodies are therefore difficult to dose and to be produced with constant quality.

Cocktails of two or more monoclonal antibodies immediately double the costs of production and,

since the efficacy and pharmacokinetic properties of each antibody differ, are difficult to formulate in order to maintain reliable administration schemes.

The present invention, however, provides monoclonal antibodies which show an advantageous binding profile to the S1-protein of SARS-CoV-2 allowing the binding and neutralization of SARS-CoV-2-activity of seven different "escape"-mutants reported up to date, as well as also the binding specifically to the "7-PM-mutant", which comprises all seven known "escape"-mutations in one molecule.

Detailed Description

In a first embodiment, the present invention pertains to a therapeutic antibody or antigen-binding fragment thereof that binds specifically to SARS-CoV-2 glycoprotein S with the amino acid sequence of SEQ ID NO: 02 and neutralizes the infectious activity of SARS-CoV-2.

In a second embodiment, the present invention pertains to a therapeutic antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes for binding to the SARS-CoV-2 glycoprotein S with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406; and a light chain variable region of the amino acid sequence in SEQ ID NO: 408.

In another embodiment, the present invention pertains to a therapeutic antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof competes for binding to the SARS-CoV-2 glycoprotein S with an antibody comprising at least one, two, three or all of the amino acid sequences of SEQ ID NO: 909 – 928 (YU505-A02); SEQ ID NO: 349 – 368 (YU536-D04); SEQ ID NOs: 389 - 408 (YU537-H11) and/or SEQ ID NO: 1305 - 1312 (STE90-C11).

In a fourth embodiment, the present invention pertains to a therapeutic antibody or antigen-binding fragment thereof, comprising a variable heavy chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 26, 46, 66, 86, 106, 126, 146, 166, 186, 206, 226, 246, 266, 286, 306, 326, 346, 366, 386, 406, 426, 446, 466, 486, 506, 526, 546, 566, 586, 606, 626, 646, 666, 686, 706, 726, 746, 766, 786, 806, 826, 846, 866, 886, 906, 926, 946, 966, 986, 1006, 1026, 1046, 1066, 1086, 1106, 1126, 1146, 1166, 1186, 1206, 1226, 1246, 1266, and 1286;

and

a light chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 48, 68, 88, 108, 128, 148, 168, 188, 208, 228, 248, 268, 288, 308, 328, 348, 368, 388, 408,

428, 448, 468, 488, 508, 528, 548, 568, 588, 608, 628, 648, 668, 688, 708, 728, 748, 768, 788, 808, 828, 848, 868, 888, 908, 928, 948, 968, 988, 1008, 1028, 1048, 1068, 1088, 1108, 1128, 1148, 1168, 1188, 1208, 1228, 1248, 1268, and 288.

In another embodiment, the present invention pertains to a therapeutic antibody or antigen-binding fragment thereof, comprising a variable heavy chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1289, 1297, 1305, 1313, 1321, 1329, 1337, 1345, 1353, 1361, 1369, 1377, 1385, 1393, 1401, 1409, 1417, 1425, 1433, 1441, 1449, 1457, 1465, 1473, 1481, 1489, 1497, 1505, 1513, 1521, 1529, 1537, 1545, 1553, 1561, 1569, 1577, 1585, 1593, 1601, 1609, 1617, 1625, 1633, 1641, 1649, 1657, 1665, 1673, 1681, 1689, 1697, 1705, 1713, and 1721;

and

a light chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1293, 1301, 1309, 1317, 1325, 1333, 1341, 1349, 1357, 1365, 1373, 1381, 1389, 1397, 1405, 1413, 1421, 1429, 1437, 1445, 1453, 1461, 1469, 1477, 1485, 1493, 1501, 1509, 1517, 1525, 1533, 1541, 1549, 1557, 1565, 1573, 1581, 1589, 1597, 1605, 1613, 1621, 1629, 1637, 1645, 1653, 1661, 1669, 1677, 1685, 1693, 1701, 1709, 1717, and 1725.

Another aspect of the invention relates to a monoclonal antibody or an antigen-binding fragment thereof characterized by a VH region and optionally a VL region each comprising 3 CDRs designated as H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2 and L-CDR3 defining the binding specificity of the antibody or the antigen-binding antibody fragment.

The position of CDRs within a VH or VL region can be defined according to Kabat, et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). In another embodiment, the method IMGT may be used to define the CDRs (Brochet, X. et al., Nucl. Acids Res. 36, W503-508 (2008). PMID: 18503082. Giudicelli, V., Brochet, X., Lefranc, M.-P., Cold Spring Harb Protoc. 2011 Jun 1; 2011 (6). pii: pdb.prot5633. doi: 10.1101/pdb.prot5633).

The CDR-sequences defined according to Kabat et al. are depicted in SEQ ID Nos: 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 210, 230, 250, 270, 290, 310, 330, 350, 370, 390, 410, 430, 450, 470, 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870, 890, 910, 930, 950, 970, 990, 1110, 1130, 1150, 1170, 1190, 1210, 1230, 1250, 1270;

12, 32, 52, 72, 92, 112, 132, 152, 172, 192, 212, 232, 252, 272, 292, 312, 332, 352, 372, 392, 412, 432, 452, 472, 492, 512, 532, 552, 572, 592, 612, 632, 652, 672, 692, 712, 732, 752, 772,

792, 812, 832, 852, 872, 892, 912, 932, 952, 972, 992, 1112, 1132, 1152, 1172, 1192, 1212, 1232, 1252, 1272;

14, 34, 54, 74, 94, 114, 134, 154, 174, 194, 214, 234, 254, 274, 294, 314, 334, 354, 374, 394, 414, 434, 454, 474, 494, 514, 534, 554, 574, 594, 614, 634, 654, 674, 694, 714, 734, 754, 774, 794, 814, 834, 854, 874, 894, 914, 934, 954, 974, 994, 1114, 1134, 1154, 1174, 1194, 1214, 1234, 1254, 1274;

16, 36, 56, 76, 96, 116, 136, 156, 176, 196, 216, 236, 256, 276, 296, 316, 336, 356, 376, 396, 416, 436, 456, 476, 496, 516, 536, 556, 576, 596, 616, 636, 656, 676, 696, 716, 736, 756, 776, 796, 816, 836, 856, 876, 896, 916, 936, 956, 976, 996, 1116, 1136, 1156, 1176, 1196, 1216, 1236, 1256, 1276;

18, 38, 58, 78, 98, 118, 138, 158, 178, 198, 218, 238, 258, 278, 298, 318, 338, 358, 378, 398, 418, 438, 458, 478, 498, 518, 538, 558, 578, 598, 618, 638, 658, 678, 698, 718, 738, 758, 778, 798, 818, 838, 858, 878, 898, 918, 938, 958, 978, 998, 1118, 1138, 1158, 1178, 1198, 1218, 1238, 1258, 1278;

20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840, 860, 880, 900, 920, 940, 960, 980, 1000, 1120, 1140, 1160, 1180, 1200, 1220, 1240, 1260, and 1280.

The CDR-sequences defined according to IMGT are depicted in SEQ ID Nos: 1290, 1298, 1306, 1314, 1322, 1330, 1338, 1346, 1354, 1362, 1370, 1378, 1386, 1394, 1402, 1410, 1418, 1426, 1434, 1442, 1450, 1458, 1466, 1474, 1482, 1490, 1498, 1506, 1514, 1522, 1530, 1538, 1546, 1554, 1562, 1570, 1578, 1586, 1594, 1602, 1610, 1618, 1626, 1634, 1642, 1650, 1658, 1666, 1674, 1682, 1690, 1698, 1706, 1714, 1722;

1291, 1299, 1307, 1315, 1323, 1331, 1339, 1347, 1355, 1363, 1371, 1379, 1387, 1395, 1403, 1411, 1419, 1427, 1435, 1443, 1451, 1459, 1467, 1475, 1483, 1491, 1499, 1507, 1515, 1523, 1531, 1539, 1547, 1555, 1563, 1571, 1579, 1587, 1595, 1603, 1611, 1619, 1627, 1635, 1643, 1651, 1659, 1667, 1675, 1683, 1691, 1699, 1707, 1715, 1723;

1292, 1300, 1308, 1316, 1324, 1332, 1340, 1348, 1356, 1364, 1372, 1380, 1388, 1396, 1404, 1412, 1420, 1428, 1436, 1444, 1452, 1460, 1468, 1476, 1484, 1492, 1500, 1508, 1516, 1524, 1532, 1540, 1548, 1556, 1564, 1572, 1580, 1588, 1596, 1604, 1612, 1620, 1628, 1636, 1644, 1652, 1660, 1668, 1676, 1684, 1692, 1700, 1708, 1716, 1724;

1294, 1302, 1310, 1318, 1326, 1334, 1342, 1350, 1358, 1366, 1374, 1382, 1390, 1398, 1406, 1414, 1422, 1430, 1438, 1446, 1454, 1462, 1470, 1478, 1486, 1494, 1502, 1510, 1518, 1526,

1534, 1542, 1550, 1558, 1566, 1574, 1582, 1590, 1598, 1606, 1614, 1622, 1630, 1638, 1646, 1654, 1662, 1670, 1678, 1686, 1694, 1702, 1710, 1718, 1726;

1295, 1303, 1311, 1319, 1327, 1335, 1343, 1351, 1359, 1367, 1375, 1383, 1391, 1399, 1407, 1415, 1423, 1431, 1439, 1447, 1455, 1463, 1471, 1479, 1487, 1495, 1503, 1511, 1519, 1527, 1535, 1543, 1551, 1559, 1567, 1575, 1583, 1591, 1599, 1607, 1615, 1623, 1631, 1639, 1647, 1655, 1663, 1671, 1679, 1687, 1695, 1703, 1711, 1719, 1727;

1296, 1304, 1312, 1320, 1328, 1336, 1344, 1352, 1360, 1368, 1376, 1384, 1392, 1400, 1408, 1416, 1424, 1432, 1440, 1448, 1456, 1464, 1472, 1480, 1488, 1496, 1504, 1512, 1520, 1528, 1536, 1544, 1552, 1560, 1568, 1576, 1584, 1592, 1600, 1608, 1616, 1624, 1632, 1640, 1648, 1656, 1664, 1672, 1680, 1688, 1696, 1704, 1712, 1720, and 1728.

According to the present invention, a VH region or the CDRs thereof alone may constitute a complete antigen-binding site. In certain embodiments, the antibody comprises a VH region or the CDRs thereof as defined herein alone. In certain embodiments, the antibody comprises a VH region or the CDRs thereof as defined herein together with a VL region or the CDRs thereof, particularly with a VL region or the CDRs thereof as defined herein. Thus, the present invention pertains in some embodiments to an antibody or antigen-binding fragment thereof, comprising:

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 210, 230, 250, 270, 290, 310, 330, 350, 370, 390, 410, 430, 450, 470, 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870, 890, 910, 930, 950, 970, 990, 1110, 1130, 1150, 1170, 1190, 1210, 1230, 1250, and 1270;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 32, 52, 72, 92, 112, 132, 152, 172, 192, 212, 232, 252, 272, 292, 312, 332, 352, 372, 392, 412, 432, 452, 472, 492, 512, 532, 552, 572, 592, 612, 632, 652, 672, 692, 712, 732, 752, 772, 792, 812, 832, 852, 872, 892, 912, 932, 952, 972, 992, 1112, 1132, 1152, 1172, 1192, 1212, 1232, 1252, and 1272;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 34, 54, 74, 94, 114, 134, 154, 174, 194, 214, 234, 254, 274, 294, 314, 334, 354, 374, 394, 414, 434, 454, 474, 494, 514, 534, 554, 574, 594, 614, 634, 654, 674, 694, 714, 734, 754, 774, 794, 814, 834, 854, 874, 894, 914, 934, 954, 974, 994, 1114, 1134, 1154, 1174, 1194, 1214, 1234, 1254, and 1274;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence selected from the group

consisting of SEQ ID NO: 16, 36, 56, 76, 96, 116, 136, 156, 176, 196, 216, 236, 256, 276, 296, 316, 336, 356, 376, 396, 416, 436, 456, 476, 496, 516, 536, 556, 576, 596, 616, 636, 656, 676, 696, 716, 736, 756, 776, 796, 816, 836, 856, 876, 896, 916, 936, 956, 976, 996, 1116, 1136, 1156, 1176, 1196, 1216, 1236, 1256, and 1276;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 38, 58, 78, 98, 118, 138, 158, 178, 198, 218, 238, 258, 278, 298, 318, 338, 358, 378, 398, 418, 438, 458, 478, 498, 518, 538, 558, 578, 598, 618, 638, 658, 678, 698, 718, 738, 758, 778, 798, 818, 838, 858, 878, 898, 918, 938, 958, 978, 998, 1118, 1138, 1158, 1178, 1198, 1218, 1238, 1258, and 1278;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840, 860, 880, 900, 920, 940, 960, 980, 1000, 1120, 1140, 1160, 1180, 1200, 1220, 1240, 1260, and 1280.

In another embodiment, the present invention pertains to an antibody or antigen-binding fragment thereof, comprising:

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1290, 1298, 1306, 1314, 1322, 1330, 1338, 1346, 1354, 1362, 1370, 1378, 1386, 1394, 1402, 1410, 1418, 1426, 1434, 1442, 1450, 1458, 1466, 1474, 1482, 1490, 1498, 1506, 1514, 1522, 1530, 1538, 1546, 1554, 1562, 1570, 1578, 1586, 1594, 1602, 1610, 1618, 1626, 1634, 1642, 1650, 1658, 1666, 1674, 1682, 1690, 1698, 1706, 1714, and 1722;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1291, 1299, 1307, 1315, 1323, 1331, 1339, 1347, 1355, 1363, 1371, 1379, 1387, 1395, 1403, 1411, 1419, 1427, 1435, 1443, 1451, 1459, 1467, 1475, 1483, 1491, 1499, 1507, 1515, 1523, 1531, 1539, 1547, 1555, 1563, 1571, 1579, 1587, 1595, 1603, 1611, 1619, 1627, 1635, 1643, 1651, 1659, 1667, 1675, 1683, 1691, 1699, 1707, 1715, and 1723;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1292, 1300, 1308, 1316, 1324, 1332, 1340, 1348, 1356, 1364, 1372, 1380, 1388, 1396, 1404, 1412, 1420, 1428, 1436, 1444, 1452, 1460, 1468, 1476, 1484, 1492, 1500, 1508, 1516, 1524, 1532, 1540, 1548, 1556, 1564, 1572, 1580, 1588, 1596, 1604, 1612, 1620, 1628, 1636, 1644, 1652, 1660, 1668, 1676, 1684, 1692, 1700, 1708, 1716, and 1724;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence selected from the group

consisting of SEQ ID NO: 1294, 1302, 1310, 1318, 1326, 1334, 1342, 1350, 1358, 1366, 1374, 1382, 1390, 1398, 1406, 1414, 1422, 1430, 1438, 1446, 1454, 1462, 1470, 1478, 1486, 1494, 1502, 1510, 1518, 1526, 1534, 1542, 1550, 1558, 1566, 1574, 1582, 1590, 1598, 1606, 1614, 1622, 1630, 1638, 1646, 1654, 1662, 1670, 1678, 1686, 1694, 1702, 1710, 1718, and 1726;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1295, 1303, 1311, 1319, 1327, 1335, 1343, 1351, 1359, 1367, 1375, 1383, 1391, 1399, 1407, 1415, 1423, 1431, 1439, 1447, 1455, 1463, 1471, 1479, 1487, 1495, 1503, 1511, 1519, 1527, 1535, 1543, 1551, 1559, 1567, 1575, 1583, 1591, 1599, 1607, 1615, 1623, 1631, 1639, 1647, 1655, 1663, 1671, 1679, 1687, 1695, 1703, 1711, 1719, and 1727;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1296, 1304, 1312, 1320, 1328, 1336, 1344, 1352, 1360, 1368, 1376, 1384, 1392, 1400, 1408, 1416, 1424, 1432, 1440, 1448, 1456, 1464, 1472, 1480, 1488, 1496, 1504, 1512, 1520, 1528, 1536, 1544, 1552, 1560, 1568, 1576, 1584, 1592, 1600, 1608, 1616, 1624, 1632, 1640, 1648, 1656, 1664, 1672, 1680, 1688, 1696, 1704, 1712, 1720, and 1728.

In certain embodiments, the antibody or antigen-binding fragment thereof comprises a VH region comprising the H-CDR1 of SEQ ID NO:10, the H-CDR2 of SEQ ID NO:12, and the H-CDR3 of SEQ ID NO:14, and optionally a VL region comprising the L-CDR1 of SEQ ID NO:16, the L-CDR2 of the SEQ ID NO:18 and the L-CDR3 of the SEQ ID NO:20; and so forth as depicted in table 1:

Table 1: Composition of certain antibody or antigen-binding fragments thereof

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
10	12	14	16	18	20
30	32	34	36	38	40
50	52	54	56	58	60
70	72	74	76	78	80

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
90	92	94	96	98	100
110	112	114	116	118	120
130	132	134	136	138	140
150	152	154	156	158	160
170	172	174	176	178	180
190	192	194	196	198	200
210	212	214	216	218	220
230	232	234	236	238	240
250	252	254	256	258	260
270	272	274	276	278	280
290	292	294	296	298	300
310	312	314	316	318	320
330	332	334	336	338	340
350	352	354	356	358	360
370	372	374	376	378	380

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
390	392	394	396	398	400
410	412	414	416	418	420
430	432	434	436	438	440
450	452	454	456	458	460
470	472	474	476	478	480
490	492	494	496	498	500
510	512	514	516	518	520
530	532	534	536	538	540
550	552	554	556	558	560
570	572	574	576	578	580
590	592	594	596	598	600
610	612	614	616	618	620
630	632	634	636	638	640
650	652	654	656	658	660
670	672	674	676	678	680

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
690	692	694	696	698	700
710	712	714	716	718	720
730	732	734	736	738	740
750	752	754	756	758	760
770	772	774	776	778	780
790	792	794	796	798	800
810	812	814	816	818	820
830	832	834	836	838	840
850	852	854	856	858	860
870	872	874	876	878	880
890	892	894	896	898	900
910	912	914	916	918	920
930	932	934	936	938	940
950	952	954	956	958	960
970	972	974	976	978	980

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
990	992	994	996	998	1000
1010	1012	1014	1016	1018	1020
1030	1032	1034	1036	1038	1040
1050	1052	1054	1056	1058	1060
1070	1072	1074	1076	1078	1080
1090	1092	1094	1096	1098	1100
1110	1112	1114	1116	1118	1120
1130	1132	1134	1136	1138	1140
1150	1152	1154	1156	1158	1160
1170	1172	1174	1176	1178	1180
1190	1192	1194	1196	1198	1200
1210	1212	1214	1216	1218	1220
1230	1232	1234	1236	1238	1240
1250	1252	1254	1256	1258	1260
1270	1272	1274	1276	1278	1280

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
1290	1291	1292	1294	1295	1296
1298	1299	1300	1302	1303	1304
1306	1307	1308	1310	1311	1312
1314	1315	1316	1318	1319	1320
1322	1323	1324	1326	1327	1328
1330	1331	1332	1334	1335	1336
1338	1339	1340	1342	1343	1344
1346	1347	1348	1350	1351	1352
1354	1355	1356	1358	1359	1360
1362	1363	1364	1366	1367	1368
1370	1371	1372	1374	1375	1376
1378	1379	1380	1382	1383	1384
1386	1387	1388	1390	1391	1392
1394	1395	1396	1398	1399	1400
1402	1403	1404	1406	1407	1408

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
1410	1411	1412	1414	1415	1416
1418	1419	1420	1422	1423	1424
1426	1427	1428	1430	1431	1432
1434	1435	1436	1438	1439	1440
1442	1443	1444	1446	1447	1448
1450	1451	1452	1454	1455	1456
1458	1459	1460	1462	1463	1464
1466	1467	1468	1470	1471	1472
1474	1475	1476	1478	1479	1480
1482	1483	1484	1486	1487	1488
1490	1491	1492	1494	1495	1496
1498	1499	1500	1502	1503	1504
1506	1507	1508	1510	1511	1512
1514	1515	1516	1518	1519	1520
1522	1523	1524	1526	1527	1528

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
1530	1531	1532	1534	1535	1536
1538	1539	1540	1542	1543	1544
1546	1547	1548	1550	1551	1552
1554	1555	1556	1558	1559	1560
1562	1563	1564	1566	1567	1568
1570	1571	1572	1574	1575	1576
1578	1579	1580	1582	1583	1584
1586	1587	1588	1590	1591	1592
1594	1595	1596	1598	1599	1600
1602	1603	1604	1606	1607	1608
1610	1611	1612	1614	1615	1616
1618	1619	1620	1622	1623	1624
1626	1627	1628	1630	1631	1632
1634	1635	1636	1638	1639	1640
1642	1643	1644	1646	1647	1648

VH-region			and, optionally, a VL-region		
H-CDR1, SEQ ID NO.:	H-CDR2, SEQ ID NO.:	H-CDR3, SEQ ID NO.:	L-CDR1, SEQ ID NO.:	L-CDR2, SEQ ID NO.:	L-CDR3, SEQ ID NO.:
1650	1651	1652	1654	1655	1656
1658	1659	1660	1662	1663	1664
1666	1667	1668	1670	1671	1672
1674	1675	1676	1678	1679	1680
1682	1683	1684	1686	1687	1688
1690	1691	1692	1694	1695	1696
1698	1699	1700	1702	1703	1704
1706	1707	1708	1710	1711	1712
1714	1715	1716	1718	1719	1720
1722	1723	1724	1726	1727	1728

The terms "SARS-CoV-2 glycoprotein S", "S protein", "homotrimeric S protein", "spike protein" or "SARS-CoV-2 spike glycoprotein" and "SARS-CoV-2 S protein", as used herein, all refer to the same protein having the nucleic acid sequence shown in SEQ ID NO: 01 and the amino acid sequence of SEQ ID NO: 02, or a substantially similar variant thereof, or a biologically active fragment thereof.

SARS-CoV-2 can be divided into different strains. According to their genotype and distribution the strains may be divided into the following subgroups: The "L-Lineage" comprises WH 2019/12/30.h, Thailand 2020/01/13.a, Japan 2020/01/25.a, TW 2020/02/05.a, WH 2020/01/02.a, WW2020/01/02.b, USA-2020/01/27.a, FS 2020/01/22.c, FS-2020/01/22.b, USA

2020/01/31.a, GZ_2020/01/22.a, Japan_2020/01/29.b, WH 2020/01/01.c, GO-2020/01/17.a, GD-2020/01/22.a, GD-020/01/18.a, USA_2020/01/29.a, WH 2019/12/30.i, Germany_2020/01/28.a, WH 2020/01/07.a, Nepal 2020/01/13.a, Thailand 2020/01/08.a, HZ 2020/01/20.a, Sydney 2020/01/22.a, ZJ 2020/01/16.a, WH 2019/12/30.1, Singapore 2020/01/25.a, Singapore 2020/01/23.a, GD 2020/01/23.a, France 2020/01/29.a, WH 2019/12/30.f, HZ 2020/01/19.a, WH 2019/12/30.g, WH 2019/12/30.k, USA 2020/01/29.b, FS 2020/01/22.a, USA 2020/01/29.c, USA-2020/01/29.d, SZ 2020/01/16.a, USA_2020/01/21.a, GD 2020/01/15.b, SZ_2020/01/16, WH 2019/12/30.n, WH 2019/12/30.c, JS 2020/01/19.a, WH_2019/12/26.a, ZJ 2020/01/17.a, WI, 2020/01/01.b, JX 2020/01/11.a, WH 2020/01/01.e, WH 2019/12/30.m, WH 2019/12/30.b, WH 2019/12/30.e, CQ 2020/01/23.a, WH_2020/01/01.f, WH 2020/01/01.a, WW2020/01/01.d, WH 2019/12/30.a, WH 2019/12/30.d, WH 2019/12/24.a, WH 2019/12/30.j, France 2020/01/23.a, France 2020/01/23.b, TW 2020/01/23.a, Sydney 2020/01/25.a, USA 2020/01/22.a, Australia_2020/01/25.a, Skorea_2020/01.a, WH_2019/12/31.a, France 2020/01/29.b, Singapore 2020/02/01.a, CQ_2020/01/18.a, and SD_2020/01/19.a.

The "S-Lineage" comprises SZ 2020/01/13.a, GD_2020/01/15.c, SZ_2020/01/13.a, GD-2020/01/15.a, GD 2020/01/14.a, SZ 2020/01/10.a, Japan 2020/01/31.b, Japan 2020/01/31.a, Japan 2020/01/29.a, SZ 2020/01/13.b, SZ_2020/01/11.a, USA 2020/01/28.a, SC 2020/01/15.a, USA 2020/01/23.a, Vietnam_2020/01/24.a, Korea 2020/01/25.a, WH 2020/01/05.a, Australia 2020/01/24.a, Belgium 2020/02/03.a, TW 2020/01/23.a, Australia 2020/01/28.a, Australia 2020/01/30.a, England 2020/01/29.a, England 2020/01/29.b, USA 2020/01/25.a, USA-2020/01/19.a, USA_2020/01/25.b, CQ 2020/01/21.a, USA 2020/01/22.b, YN_2020/01/17.b, and YN_2020/01/17.a.

In one embodiment the therapeutic antibody or antigen-binding fragment thereof is preferred which binds specifically to at least one of the SARS-CoV-2 glycoprotein S from the above mentioned strains. In yet another embodiment it binds specifically more preferably to the SARS-CoV-2 glycoprotein S of one of the most abundant strains. In yet another embodiment the therapeutic antibody or antigen-binding fragment thereof binds specifically to the SARS-CoV-2 glycoproteins S of at least two, at least five, at least 10, at least 20, at least 30 strains. In a most preferred embodiment it binds specifically to the SARS-CoV-2 glycoproteins S of all SARS-CoV-2-strains listed above.

In another embodiment the therapeutic antibody or antigen-binding fragment thereof binds preferably to the SARS-CoV-2 glycoproteins S of at least one strain of the "L-lineage" as listed above. In yet another embodiment the therapeutic antibody or antigen-binding fragment thereof binds preferably to the SARS-CoV-2 glycoproteins S of at least one strain of the "P-strains" as listed above.

The homotrimeric S protein is a class I fusion protein which mediates the receptor binding and membrane fusion between the virus and host cell. The S1 subunit forms the head of the spike and has the receptor binding domain (RBD). The S2 subunit forms the stem which anchors the spike in the viral envelope and on protease activation enables fusion. In addition, the membrane bound host protease TMPRSS2 is responsible for S protein priming by cleavage of the furin site between S1 and S2 as well as the S2' site for proteolytic activation, conformational change and viral entry.

The "S1 subunit", as used herein, has the nucleic acid sequence shown in SEQ ID NO: 03 and the amino acid sequence of SEQ ID NO: 04, or a substantially similar variant thereof, or a biologically active fragment thereof.

The "receptor binding domain" (RBD) or "RBD" or "S1-RBD", as used herein, has the nucleic acid sequence shown in SEQ ID NO: 05 and the amino acid sequence of SEQ ID NO: 06, or a substantially similar variant thereof, or a biologically active fragment thereof.

SARS-CoV-2 is believed to bind via the RBD to ACE2, a surface protein of the host cell.

The term "Angiotensin-converting enzyme 2" or "ACE2", as used herein, refers to the human version of Angiotensin-converting enzyme 2 having the nucleic acid sequence shown in SEQ ID NO: 07 and the amino acid sequence of SEQ ID NO: 08, or a biologically active fragment thereof.

For reference the following SEQ ID NOs pertain to the following molecules:

SEQ ID NO: 01 – DNA-sequence of full-length SARS-CoV-2 glycoprotein S (Spike)

SEQ ID NO: 02 – Protein-sequence of full-length SARS-CoV-2 glycoprotein S (Spike)

SEQ ID NO: 03 – DNA-sequence of full-length of S1-subunit of the SARS-CoV-2 glycoprotein S

SEQ ID NO: 04 – Protein-sequence of full-length of S1-subunit of the SARS-CoV-2 glycoprotein S

SEQ ID NO: 05 – DNA-sequence of full-length of receptor binding domain (RBD) of the S1-subunit of SARS-CoV-2 glycoprotein S

SEQ ID NO: 06 – Protein-sequence of full-length of receptor binding domain (RBD) of the S1-subunit of SARS-CoV-2 glycoprotein S

SEQ ID NO: 07 – DNA-sequence of full-length human ACE2

SEQ ID NO: 08 – Protein-sequence of full-length von human ACE2

In another embodiment the therapeutic antibody or antigen-binding fragment thereof specifically binds preferably to the version of the SARS-CoV-2 glycoproteins S as depicted in SEQ ID NO: 02 as well as at least one, two, three, four, five, six, or preferably all of the “escape”-mutants mentioned herein: S1-V367F, S1-N439K, S1-G476S, S1-V483A, S1-E484K, S1-G485R, S1-F486V and S1-7PM (SEQ ID NO: 1730). (The numbering of the residues correspond to the numbering used in the SWISS-MODEL workspace (YP_009724390.1);(UniProt ID P0DTC2).

The use of the disclosed point mutations, preferably to the S1-7PM-mutant, for screening potential therapeutic antibodies, has the technical effect that antibodies are selected which bind not only to the “wild-type” virus, but also to other viral strains which have already developed or may develop mutations in near future. Thus, the therapeutic antibody or antigen-binding fragment thereof of the present invention is able to recognize both “wild-type” as well as mutated versions of the SARS-CoV-2-virus. Consequentially, patients with “wild-type” virus populations only, with variable virus populations, or viral populations only comprising mutated viruses, may still be treated with the inventive antibodies.

Additionally, a second effect is that it is more difficult for the virus to develop resistance to said antibodies, since the virus needs to mutate further in order to “escape” the binding of the therapeutic antibody or antigen-binding fragment thereof. As such no other strategies, like for example antibody-cocktails comprising two or more different antibodies need to be applied.

Thus, the antibodies of the present invention provide a “one fits all” solution, which is more cost-effective and results in a simplified formulation scheme and market approval process.

The sequence alignment between the “wild-type” RBD-sequence (SEQ ID NO: 1830) and the S1-7PM mutation (SEQ ID NO: 1829) is depicted below, the respective point mutations are highlighted in bold:

319 RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYS**FL** 368

319 RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYS**VL** 368

369 YNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKI 418

369 YNSASFSTFKCYGVSPSTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKI 418

419 ADYNYKLPDDFTGCVIAWNS**KN**LDSKVGGNYNLYRLFRKSNLKPFERDI 468

419 ADYNYKLPDDFTGCVIAWNS**NN**LDSKVGGNYNLYRLFRKSNLKPFERDI 468

469 STEIYQ**AS**STPCNG**AKRV**NCYFPLQSYGFQPTNGVGYQPYRVVLSFELL 518

469 STEIYQ**AG**STPCNG**VEGF**NCYFPLQSYGFQPTNGVGYQPYRVVLSFELL 518

519 HAPATVCGPKKSTNLVKNKCVNFN 542 (SEQ ID NO: 1829)

519 HAPATVCGPKKSTNLVKNKCVNFN 542 (SEQ ID NO: 1830)

The term "antibody", as used herein, is intended to refer any antigen binding molecule, in some embodiments to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "VH") and a heavy chain constant region (comprised of domains CH1, CH2 and CH3). Each light chain is comprised of a light chain variable region ("LCVR" or "VL") and a light chain constant region (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" (CDR), interspersed with regions that are more conserved, termed "framework regions" (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Substitution of up to one, up to two or up to three amino acid residues per CDR, of up to two, up to three or up to four amino acid residues within all three CDRs of one light or heavy chain or omission of one or more CDRs is also possible.

In one embodiment up to one, up to two or up to three amino acid residues are substituted in H-CDR1. In another embodiment up to one, up to two or up to three amino acid residues are substituted in H-CDR2. In yet another embodiment up to one, up to two or up to three amino acid residues are substituted in H-CDR3. In one embodiment up to one, up to two or up to three amino acid residues are substituted in L-CDR1. In another embodiment up to one, up to two or up to three amino acid residues are substituted in L-CDR2. In yet another embodiment up to one, up to two or up to three amino acid residues are substituted in L-CDR3.

Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding.

If a CDR or amino acid residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences.

In one embodiment up to one, up to two or up to three amino acid residues are omitted in H-CDR1. In another embodiment up to one, up to two or up to three amino acid residues are omitted in H-CDR2. In yet another embodiment up to one, up to two or up to three amino acid residues are omitted in H-CDR3. In one embodiment up to one, up to two or up to three amino acid residues are omitted in L-CDR1. In another embodiment up to one, up to two or up to three amino acid residues are omitted in L-CDR2. In yet another embodiment up to one, up to two or up to three amino acid residues are omitted in L-CDR3.

Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

In some embodiments also in other parts of the antibody or antigen-fragment thereof amino acids may be exchanged by conservative amino acid substitution. Such a sequence variant, which differs from another sequence only by conservative amino acid substitution(s), is called herein "substantially similar variant".

A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., similar charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art.

Examples of groups of amino acids that have side chains with similar chemical properties, and whose substitution for each other constitutes conservative substitutions, include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Science 256: 1443 45. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

Sequence similarity for polypeptides is typically measured using sequence analysis software.

Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions.

For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof.

Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra).

Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, e.g., Altschul et al. (1990) J. Mol. Biol. 215: 403 410 and (1997) Nucleic Acids Res. 25:3389 402.

The term "sequence identity" when referring to a nucleic acid or antigen-binding fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed.

As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, at least 98% or at least 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

In some embodiments, the invention is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific

antibody, or an antigen-binding fragment thereof. In some embodiments, the isolated antigen binding protein is a Fab fragment, a Fab' fragment, a F(ab')₂ fragment, a Fv fragment, a di-
abody, a nanobody or a single chain antibody molecule. In some embodiments, the isolated an-
tigen binding protein is of the IgG1-, IgG2- IgG3- or IgG4-type. In some embodiments the anti-
body may be of IgA-, IgD-, IgE- or IgM-type.

In one embodiment the invention is a monoclonal antibody of IgG1-Type. In another embodi-
ment the invention is a scFV-fragment.

The term "human antibody", as used herein, is intended to include antibodies having variable
and constant regions derived from human germline immunoglobulin sequences. The human
mAbs of the invention may include amino acid residues not encoded by human germline immu-
noglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in
vitro or by somatic mutation in vivo), for example in the CDRs.

The term "therapeutic" antibody or antigen-binding fragment thereof, as used herein, is in-
tended to differentiate the antibodies of this invention from "regular" antibodies or fragments
thereof. Regular antibodies may also bind to the target, but usually they show low affinity, or low
specificity, or neutralizing effect, or a combination thereof, to the target, i.e. SARS-CoV-2 glyco-
protein S. Regular antibodies may also show other undesired features, such as dimerization or
multimerization. Such regular antibodies may be suitable for certain scientific applications, such
as assays. However, such regular antibodies must not be used for therapeutic purposes, which
necessitate therapeutic antibodies with high affinity, high specificity and/or high neutralizing ef-
fects; and which are free of undesired features, such as agglutination, such as dimerization or
multimerization, which may lead to an undesired host-response, for example immune-response,
after treatment with such antibodies and, therefore, such antibodies are normally not admitted
for therapeutic use by the regulatory health authorities.

The difference in appearance of a therapeutic antibody and a regular antibody as measured by
SEC-HPLC is shown in Figures 1a and 1b. Figure 1a depicts a therapeutic antibody which is
nearly 100% monomeric free of agglutination, whereas Figure 1b depicts a regular antibody with
dimeric and multimeric peaks. The integral of the curves depicts the amount of monomeric, di-
meric and multimeric antibodies. Please note that agglutinations elute quicker from SEC-HPLC
than monomers.

In some embodiments, the therapeutic antibodies or antigen binding fragment thereof should be
monomeric antibodies with a contamination with agglutinations of less than 10 mol-%, prefera-
bly less than 5 mol-%, more preferably less than 2 mol-%, most preferred less than 1 mol-% of
agglutinations such as dimeric and/or multimeric antibodies as measured with size-exclusion
chromatography (SEC)-HPLC with PBS at 20°C according to standard protocol.

In some embodiments the contamination with agglutinations is “absent”, that is, not measurable with SEC-HPLC (i.e. is between 0 – 0.25 mol-%).

In another embodiment the therapeutic antibodies or antigen binding fragment thereof are monomeric antibodies are defined by a “contamination ratio” which is the ratio of the integrals of the HPLC-peak as measured with size-exclusion chromatography (SEC)-HPLC denoting the monomeric antibody (int-mon) and the integrals of the agglutination HPLC-peaks as measured with size-exclusion chromatography (SEC)-HPLC (int-mult) as depicted in Formula I:

Formula I:

$$\frac{int - mult}{int - mon} = contamination\ ratio$$

In another embodiment the therapeutic antibodies or antigen binding fragment thereof possesses a “contamination ratio” less than 0.2, more preferred of less than 0.1, even more preferred of less than 0.05, even more preferred of less than 0.033, even more preferred of less than 0.02.

In some embodiments the therapeutic antibodies or antigen binding fragment thereof is monomeric antibodies free of any contamination with dimeric and/or multimeric antibodies as measured with size-exclusion chromatography (SEC)-HPLC with PBS at 20°C according to standard protocol..

Dimeric and multimeric antibodies, also summarized with the term “agglutination” may provoke unwanted side-effects during therapy and must be reduced or, preferably, completely avoided.

Without being bound to theory, the formation of antibody agglutinations during manufacturing, storage and distribution, the exposure of the antibody to different stress conditions such as mechanical stress, light exposure, temperature differences, pH variations and/or contact to different surface materials, may be the result of mal-designed antibodies (e.g. unfavorable charge distribution, unpaired cysteine-residues, unfavorable hydrophobic patches and the like).

In some embodiments of the present invention the therapeutic antibodies or antigen binding fragment thereof should possess a low “stickiness”. The term “stickiness” means the ability of the antibody to aggregate unspecifically with other antibodies. This “stickiness” can be measured by a “specificity ELISA” as outlined in more detail in the examples-section.

Preferred antibodies show a mean stickiness against a control-antibody (for example against Avelumab) of less than 0.9, preferably of less than 0.8, more preferably of less than 0.7, even more preferably of less than 0.6; most preferably of less than 0.5 when measured in a specificity

ELISA.

In some embodiments the therapeutic antibodies and antigen-binding fragments thereof possess a stickiness of between 0.4 and 0.75 when measured in a specificity ELISA in comparison to Avelumab.

Fc mediated effector functions support anti-viral function of antibodies, but they can also cause antibody dependent enhancement (ADE) and other severe adverse effect such as cytokine storms and hyperinflammation.

Potential adverse effects of active Fc portion of IgG1 are:

- ADE: FcγR binding on phagocytes or other cells may re-target infection (It is possible that non-neutralizing anti-virus antibodies and/or suboptimal concentrations may actually enhance the infection).
- Hyperinflammation and/or cytokine storm: FcγR binding and activation on immune cells may lead to enhancement of inflammatory events associated with the viral infection or underlying diseases.
- Complement activation may lead to endotheliopathy, hypercoagulability, hyperinflammation and cytokine storm.

COVID-19 is associated with large number of risk factor, mostly underlying diseases (ca. 90% of severe cases in the US). Therefore, antibody drugs with an active Fc portion may cause adverse effects in these cases and safety measures need to be included into the drug and therapy design. Thus, in some embodiments of the present invention, the constant region (Fc-region) of the therapeutic antibody and/or antigen-binding fragment thereof was designed or further amended in order prevent an antibody-dependent enhancement (ADE) and/or cytokine storm syndrome (CSS).

The term "antibody-dependent enhancement" (ADE), sometimes less precisely called immune enhancement or disease enhancement, is a phenomenon in which binding of a virus to antibodies enhances its entry into host cells, and sometimes also its replication.

It is believed that some host cells lack the usual receptors on their surfaces that the virus uses to gain entry, but they have Fc receptors that bind to one end of antibodies. The virus binds specifically to the antigen-binding site at the other end, and in this way gains entry to and infects the host cell, potentially increasing the severity of the disease.

The "cytokine storm", also called cytokine storm syndrome (CSS), is a bodily reaction in human

immune systems in which the innate immune system releases a large number of cytokines, potentially overwhelming the body and possibly leading to acute respiratory distress syndrome (ARDS) and even fatality. It is believed that many severe episodes of COVID-19 are related at least partially to the cytokine storm syndrome.

The respective modifications which were introduced in the constant region in order to produce "silent Fc"-regions are depicted in table 2. In some embodiments already one, two or three of the depicted modifications may be introduced in order to achieve sufficient silencing.

Table 2: Modifications in Fc-region in order to produce silenced Fc.

Fc mutation	Effect on FcγRs Ratio to wt mean (SD)				Effect on complement	FcRn	References and remarks
	FcγR1	FcγR 2A	FcγR 2B	FcγR 3A			
Numbering [3]							
E233P	0.12 (0.06)	0.08 (0.06)	0.12 (0.01)	0.04 (0.02)	?	0.54 (0.20)	[1] from IgG2 other mutations at this position reduce FcγR binding (e.g. LALA)
L234V					?		
L235A					?		
ΔG236					?		
D265G	0.16 (0.05)	0.07 (0.01)	0.13 (0.05)	0.09 (0.06)	Reduced C1q binding?	1.23 (0.14)	[1] D265A: 44.7% 1-3 terminal Gal and higher sialation vs wt: 33.9%
A327Q	0.6 (0.12)	0.13 (0.03)	0.14 (0.03)	0.06 (0.01)	?	0.97	[1] similar A327G [2]
A330S	?	?	?	?	Decreased C1q	?	[2] from IgG4. complement binding
Total	0	0	0	0	0		

SD =Standard deviation; wt =wildtype IgG1-Fc

[1] Shields et al.

[2] Armour, KL, Clark, MR, Hadley, AG, and Williamson, LM. Recombinant human IgG molecules lacking Fcγ receptor I binding and monocyte triggering activities Eur. J. Immunol. 1999. 29: 2613–2624.

[3] Kabat, EA, Wu, TT, Perry, HM, Gottesman, KS and Foeller, C, Sequences of proteins of immunological interest. US Department of Health and Human Services, NIH, Bethesda 1991. (EU Numbering system).

It is obvious to the person skilled in the art, that, whereas regular antibodies may not be used for therapeutic use i.e. *in vivo* application and/or treatment of patients because of the above-mentioned reasons, therapeutic antibodies are suitable also for non-therapeutic use i.e. *in vitro* assays, and the like.

The term "antibody fragment", as used herein, refers to one or more fragments of an antibody that retains the ability to specifically bind to S1-protein and/or RBD.

An "antibody fragment" may include a Fab fragment, a F(ab')₂ fragment, a Fv fragment (including scFv-fragments), a dAb fragment, a fragment containing a CDR, or an isolated CDR.

The present invention generally relates to antibodies or antigen-binding fragments thereof capable of specifically blocking and/or inhibiting the interaction between the SARS-CoV-2 spike protein and the ACE receptor, particularly the human ACE receptor, as well as combinations comprising two or more different antibodies or antigen-binding fragments. Specific amino acid sequences of relevant antibody regions are described in the enclosed sequence listing.

The term "specifically blocking and/or inhibiting the interaction between the SARS-CoV-2 spike protein and the ACE receptor" as used herein, refers to the inhibition of the binding between the S1 domain of the spike protein and an ACE positive cell, particularly when determined as described in the present examples. In particular embodiments, the antibody binds specifically to the S1 domain of the spike protein.

More particularly, the present invention relates to an antibody or antigen-binding fragment thereof capable of specifically blocking and/or inhibiting the interaction between the SARS-CoV-2 spike protein and the human ACE receptor defined by one or more complementarity-determining regions (CDRs) and/or by variable heavy chain (VH) and optionally variable light chain (VL) regions comprising said CDRs or related sequences.

In some embodiments of the present invention, the antibodies or antigen-binding fragments of the invention are neutralizing.

A "neutralizing antibody", as used herein (or an "antibody that neutralizes the infectious activity

of SARS-CoV-2"), is intended to refer to an antibody whose binding specifically to the SARS-CoV-2 glycoprotein S, more preferably the S1-subunit and/or RBD results in inhibition of virus infectivity.

The term "virus infectivity" or "viral infection" as used herein means any viral activity in the host. Viral infection occurs when a host's body is invaded by SARS-CoV-2, and infectious virus particles (virions) attach to and enter susceptible host cells. Thus, the term "virus infectivity" includes viral activities, such as the entry of virus particles into host cells, the replication of the virus within the host cell, the lysis of the host cell and spreading of the virus to further cells. However, "inhibition of virus infectivity" can also mean the prevention, reduction or healing of any disease or condition resulting from a SARS-CoV-2 infection, especially symptoms associated with COVID-19. Such a disease or condition includes one or more symptoms selected from the list comprising fever, cough, fatigue, shortness of breath, loss of smell and taste, pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD) with diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC), leukoerythroblastic reaction and microvesicular steatosis in the liver and any combination thereof.

The terms "neutralizing" and "blocking" or "inhibition" are not to be confused with each other. Antibodies exist, which bind to the S1-subunit and/or RBD in such a way that they block/inhibit the binding of the spike-protein to the ACE2-receptor, however, they still may not neutralize the infectious activity of SARS-CoV-2, and, thus, possess no neutralizing activity.

Only if also at least one infectious activity of the SARS-CoV-2-virus is inhibited, the term "neutralizing" is used herein. This inhibition of the infectious activity can be assessed by measuring one or more indicators of viral activity by one or more of several standard *in vitro* or *in vivo* assays known in the art, such as for example the SARS-CoV-2 VERO E6 neutralizing assay, wherein VERO E6 cells are infected with live SARS-CoV-2 virus and the neutralization of the antibody or antigen-binding fragment thereof is measured by the retention of confluence after certain time points.

VERO E6 cells are commonly used for *in vitro* studies of the severe acute respiratory syndrome-associated coronavirus (SARS-CoV) and for antiviral evaluation purposes. In these tests the SARS-CoV growth kinetics in VERO E6 cells is used in order to elucidate the mechanism of antiviral activity of selective antiviral agents. The growth kinetics of SARS-CoV in VERO E6 cells

can be measured qualitatively (or half-quantitatively) by cell-confluence measurement as outlined above and/or quantitatively by intra- and extracellular viral RNA load as well as extracellular virus yield at different time points post-infection.

Without virus-neutralizing agents, at 12 h post-infection, the intracellular viral RNA load is about 3×10^2 -fold higher than at the time of infection, and the extracellular viral RNA load is increased by a factor of about 2×10^3 . Intracellular viral RNA levels started to rise at 6 h post-infection. One hour later (at 7 h post-infection), the levels of extracellular SARS-CoV RNA also begins to rise. This is corroborated by the fact that infectious progeny SARS-CoV also first appeared in the supernatant between 6 and 7 h post-infection. At 12 h post-infection, SARS-CoV reaches titers in the supernatant of 5.2×10^3 CCID₅₀/ml.

Thus, a “neutralizing antibody or antigen-binding fragment thereof” is any antibody or antigen-binding fragment thereof where confluence in a SARS-CoV-2 VERO E6 neutralizing assay in % at 4 dpi is at least 80%, more preferred at least 85%, even more preferred at least 90%, even more preferred at least 95%, most preferred at least 98% after incubation with a mix comprising at least medium, active SARS-CoV-2 and the neutralizing antibody or antigen-binding fragment thereof. In some embodiments, e.g. see example 9, the viral strain SARS-CoV-2/Münster/FI 110320/1/2020 was used. The skilled person is aware that it is possible to use also other SARS-CoV-2 viral strains with similar infectivity to VERO E6 cells.

In case of some embodiments the confluence in SARS-CoV-2 VERO E6 neutralizing assay in % at 4 dpi is between 98% and up to 100%, e.g. the same confluence as visible for non-infected VERO E6 – cells at the same time-point.

In some embodiments the time-point is chosen about 3.5 days after the first incubation with SARS-CoV-2, in other embodiments 3 days and 18 hours; in further embodiments 4 days, 5 days and up to 6 days, or 7 days.

In another embodiment, a “neutralizing antibody or antigen-binding fragment thereof” is any antibody or antigen-binding fragment thereof where extracellular viral RNA load in a SARS-CoV-2 VERO E6 neutralizing assay is reduced by at least 50% as compared to infected cells without treatment with a “neutralizing antibody or antigen-binding fragment thereof”, more preferred at least 75%, even more preferred at least 80%, even more preferred at least 90%, even more preferred at least 95%, even more preferred at least 99%, most preferred 99.9%.

In another embodiment, a “neutralizing antibody or antigen-binding fragment thereof” is any antibody or antigen-binding fragment thereof where intracellular viral RNA levels in a SARS-CoV-2 VERO E6 neutralizing assay is reduced by at least 50% as compared to infected cells without treatment with a “neutralizing antibody or antigen-binding fragment thereof”, more preferred at

least 75%, even more preferred at least 80%, even more preferred at least 90%, even more preferred at least 95%, even more preferred at least 99%, most preferred 99.9%.

In yet another embodiment, a "neutralizing antibody or antigen-binding fragment thereof" is any antibody or antigen-binding fragment thereof where at 12h post infection SARS-CoV-2 titers in the supernatant in a SARS-CoV-2 VERO E6 neutralizing assay is reduced by at least 50% as compared to infected cells without treatment with a "neutralizing antibody or antigen-binding fragment thereof", more preferred at least 75%, even more preferred at least 80%, even more preferred at least 90%, even more preferred at least 95%, even more preferred at least 99%, most preferred 99.9% and/or the virus titration by cell culture infectious dose 50% assay (CCID₅₀/ml) is less than 5×10^2 CCID₅₀/ml, more preferred less than 1×10^2 CCID₅₀/ml, 5×10^1 CCID₅₀/ml, or 1×10^1 CCID₅₀/ml.

Thus, in some embodiments the "antibody that neutralizes S1-subunit and/or RBD activity" inhibits or reduces the interaction, in some embodiments the binding of the S1-subunit and/or RBD to the ACE2-protein on the host cell surface and results in prevention or reduction of entry of virus particles into the host cell.

In some embodiments, the neutralizing ability in vitro is described in terms of an IC₅₀ value. In case of in vivo neutralizing ability the EC₅₀ is preferred. Thus, the IC₅₀ value indicates the concentration of an inhibitor which is necessary to block 50% of an enzyme, a cell, a cell receptor or a microorganism (in general: "targets") in vitro. The EC₅₀ value indicates this required concentration in vivo, i.e. for the binding ability to S1/RBD.

Thus, in yet another embodiment, a "neutralizing antibody or antigen-binding fragment thereof" is any antibody or antigen-binding fragment thereof where the IC₅₀ as measured in the VERO E6 cell assay is less than 0.2 µg/ml, less than 0.1 µg/ml, more preferred less than 0.05 µg/ml even more preferred less than 0.04 µg/ml, even more preferred less than 0.036 µg/ml, even more preferred less than 0.030 µg/ml, most preferred less than 0.026 µg/ml as measured in an infectious VERO E6 cell assay.

In another embodiment the neutralizing effect of the therapeutic antibody (IC₅₀) is about 0.03672 µg/ml, in yet another embodiment is about 0.03599 µg/ml as measured in an infectious VERO E6 cell assay, in yet another embodiment is about 0.02539 µg/ml as measured in an infectious VERO E6 cell assay.

In another embodiment the neutralizing effect of the therapeutic antibody (IC₅₀) is less than 1.0 nM, preferably less than 0.85 nM, more preferably less than 0.60 nM, most preferably less than 0.50 nM as measured in an infectious VERO E6 cell assay.

Further examples for IC₅₀-values of therapeutic antibodies of the present invention are depicted in figures 16 and 17.

In one embodiment only antibodies with said excellent low IC₅₀-values of less than 0.06 µg/ml even more preferred less than 0.049 µg/ml, even more preferred less than 0.036 µg/ml, most preferred less than 0.026 µg/ml as measured in an infectious VERO E6 cell assay, can be considered “therapeutic antibodies”, since only these antibodies facilitate sufficient neutralizing activity within a patient.

In certain embodiments, the antibody or antigen-binding fragment has an EC₅₀ value of about 20 nM or less on RBD, of about 10 nM or less, or of about 5 nM or less for the S1 domain of the spike domain, particularly when determined as described in the present example 9.

A low IC₅₀ and/or EC₅₀ may result in the beneficial effect that smaller antibody dosages can be administered. This has an effect on the frequency, viscosity and/or volume of the pharmaceutical composition which needs to be administered.

For example an antibody with a less preferred higher IC₅₀ and/or EC₅₀-value may necessitate a higher frequency of treatment, e.g. instead of once or twice during the whole course of the disease, maybe each day or several times per day.

It may also result in the effect that the volume per treatment needs to be so high that not a normal syringe or auto-injector, but a large volume device, i.e. pump-device or perfusion needs to be used.

It may also result in the effect that the administration device needs to be composed of less-breakable material, since due to the high viscosity of the high concentrated antibody composition needs to be applied with elevated pressure.

Finally, it may also result in the effect that the antibody-composition is not commercially feasible.

In certain embodiments, the antibody or antigen-binding fragment inhibits the binding specifically to the spike protein with a molar ratio of about 70:1 or less, particularly when determined as described in the present example 3.

In one embodiment YU536-D04 (SEQ ID NOs: 349 - 368) is considered such a “therapeutic antibody”, in yet another embodiment YU537-H11 (SEQ ID NOs: 389 - 408) is considered such a “therapeutic antibody”, in yet another embodiment STE90-C11 (SEQ ID NO: 1305 - 1312) is considered such a “therapeutic antibody”, in yet another embodiment YU505-A02 (SEQ ID NO: 909 - 928) is considered such a “therapeutic antibody”.

Thus, in one embodiment the therapeutic antibody or antigen-binding fragment thereof that binds specifically to SARS-CoV-2 glycoprotein S with the amino acid sequence of SEQ ID NO: 02 and neutralizes the infectious activity of SARS-CoV-2 is selected from the list consisting of:

YU505-A01 (SEQ ID NOs: 889 – 908), YU505-A02 (SEQ ID NOs: 909 - 928), YU505-F05 (SEQ ID NOs: 1149 - 1168), YU534-C12 (SEQ ID NOs: 169 - 188), YU534-D09 (SEQ ID NOs: 509 - 528), YU535-A02 (SEQ ID NOs: 189 - 208), YU537-H08 (SEQ ID NOs: 769 - 788), YU537-H11 (SEQ ID NOs: 389 - 408), YU534-C09 (SEQ ID NOs: 149 – 168), YU536-D04 (SEQ ID NOs: 349 - 368), YU535-A02 (SEQ ID NOs: 189 - 208), YU535-C06 (SEQ ID NOs: 529 – 548) and YU505-E01 (SEQ ID NOs: 1049 – 1068).

In some embodiments, the antibodies in Table 1 above are strong neutralizers.

In some embodiments of the present invention, the antibodies or antigen-binding fragments of the invention bind specifically to the target.

The term "specifically binds" or "binds specifically" or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of less than 1×10^{-6} M, preferably less than 1×10^{-7} M, more preferably less than 1×10^{-8} M, even more preferably less than 1×10^{-9} M; most preferably less than 1×10^{-10} M or less (e.g., a smaller K_D denotes a tighter binding) as measured by affinity chromatography using protein A or surface plasmon resonance, e.g. BIACORE™, biolayer interferometer (BLI), SPRi, solution-affinity ELISA. An isolated antibody or antigen-binding fragment thereof that specifically binds the S1-subunit and/or RBD may, however, still have cross-reactivity to other related antigens, such as the S1-subunit and/or RBD molecules from other species.

Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds the S1-subunit and/or RBD may, however, exhibit cross-reactivity to other antigens such as S1-subunit and/or RBD molecules from other viral species.

In some embodiments of the present invention, the antibodies or antigen-binding fragments of the invention bind with high affinity to the target.

The term " K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

The term "high affinity" antibody refers to those mAbs having a binding affinity K_D to S1-protein and/or RBD of less than 1×10^{-8} M, preferably of less than 5×10^{-9} M, preferably of less than 1×10^{-9} M, preferably of less than 1×10^{-10} M; more preferably of less than 10^{-11} M; even more

preferably 10^{-12} M or less, as measured by affinity chromatography using protein A or by surface plasmon resonance (BIAcore™), or by Bio-Layer Interferometry (BLI).

The terms "fast on rate" or " k_{on} " or " k_a " refer to an antibody or antigen-binding fragment thereof that dissociates from S1-subunit and/or RBD with a rate constant of $1 \times 10^5 \text{ Ms}^{-1}$ or more, preferably $1 \times 10^6 \text{ Ms}^{-1}$ or more, as determined by surface plasmon resonance, e.g., BIAcore™.

The terms "slow off rate" or " k_{off} " or " k_d " refer to an antibody or antigen-binding fragment thereof that dissociates from S1-subunit and/or RBD with a rate constant of $1 \times 10^{-3} \text{ s}^{-1}$ or less, preferably $1 \times 10^{-4} \text{ s}^{-1}$ or less, as determined by Bio-Layer Interferometry (BLI).

The term "Bio-Layer Interferometry (BLI)", as used herein, refers a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time.

The binding between a ligand immobilized on the biosensor tip surface and an analyte in solution produces an increase in optical thickness at the biosensor tip, which results in a wavelength shift, $\Delta\lambda$, which is a direct measure of the change in thickness of the biological layer. Interactions are measured in real time, providing the ability to monitor binding specificity, rates of association and dissociation, or concentration, with high precision and accuracy.

Only molecules binding specifically to or dissociating from the biosensor can shift the interference pattern and generate a response profile. Unbound molecules, changes in the refractive index of the surrounding medium, or changes in flow rate do not affect the interference pattern. This is a unique characteristic of bio-layer interferometry and extends its capability to perform in crude samples used in applications for protein-protein interactions, quantitation, affinity, and kinetics.

BLI-systems are commercially available such as for example Octet® QKe (Fortebio/Sartorius GmbH, Göttingen, Germany).

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system.

An "isolated antibody", as used herein, is intended to refer to an antibody or antigen-binding fragment thereof that is substantially free of other antibody or antigen-binding fragment thereof having different antigenic specificities (e.g., an isolated antibody that specifically binds the S1-subunit and/or RBD is substantially free of mAbs that specifically bind antigens other than the

S1-subunit and/or RBD).

The term "compete" when used in the context of the antibody or antigen-binding fragment thereof means competition between the reference antibody or antigen-binding fragment thereof in binding specifically to the target protein (also called "antigen", in the specific case of the invention this is the RBD and/or the S1-subunit of SARS-CoV-2 S protein) with other antigen-binding molecule.

Such antigen-binding molecule may be selected from the list of: natural ligands to the RBD and/or S1-subunit of SARS-CoV-2 S protein; including ACE2 and fragments thereof; other antibodies or antigen-binding fragments thereof; diabodies, nanobodies, anticalins, scab-proteins, single-chain antibody variable regions, single-chain fragments variables (scFv), etc.

This competition may be determined by an assay in which the reference antibody or antigen-binding fragment thereof (i.e. one of the reference antibodies mentioned hereinunder) prevents or inhibits (e.g., reduces) specific binding of another antigen-binding molecule, such as for example a test-antibody or antigen-binding fragment thereof and/or ACE2 or fragments thereof, from binding specifically to the RBD of the S1-subunit of the SARS-CoV-2 S protein.

Numerous types of competitive binding assays can be used to determine if one antigen binding protein competes with another, for example:

- solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich;
- competition assay (see, e.g., Stahli et al., 1983, Methods in Enzymology 9:242-253);
- solid phase direct biotin-avidin EIA (see, e.g., Kirkland et al., 1986, J. Immunol. 137:3614-3619)
- solid phase direct labeled assay;
- solid phase direct labeled sandwich assay (see, e.g., Harlow and Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Press);
- solid phase direct label RIA using I-125 label (see, e.g., Morel et al., 1988, Molec. Immunol. 25:7-15);
- solid phase direct biotin-avidin EIA (see, e.g., Cheung, et al., 1990, Virology 176:546-552); and direct labeled RIA (Moldenhauer et al., 1990, Scand. J. Immunol. 32:77-82).

Typically, such an assay involves the use of purified RBD and/or S1-subunit of SARS-CoV-2 S

protein as “antigen”, bound to a solid surface or cells bearing either of these, an unlabeled test antigen-binding molecule and a labeled reference antigen-binding protein, for example a reference antibody.

In one embodiment the labeled reference antigen-binding protein may be selected from a group of amino acids depicted in SEQ IDs NO: 906, 908, 926, 928, 1066, 1068, 1166, 1168, or any combination and/or antigen-binding fragment thereof.

In one embodiment the labeled “reference antibody” is selected from YU505-A02 (SEQ ID NOs: 909 - 928), YU536-D04 (SEQ ID NOs: 349 - 368), YU537-H11 (SEQ ID NOs: 389 - 408) and/or STE90-C11 (SEQ ID NOs: 1305 -1312).

In another embodiment the labeled reference antigen-binding protein may be ACE2 of human or mammalian (e.g. mouse, dog, monkey, bat, pangolin) origin, or any fragment thereof which is still able to bind to the S1-subunit of the SARS-CoV-2 glycoprotein S domain.

In yet another embodiment the labeled reference antigen-binding protein may be any molecule, protein or any fragment thereof which is able to bind to the S1-subunit of the SARS-CoV-2 glycoprotein S domain, preferably the RBD-domain of SARS-CoV-2 glycoprotein S.

Competitive inhibition is measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually the antigen binding protein is present in excess.

Antigen binding proteins identified by competition assay (competing antigen-binding molecules) include antigen binding proteins binding specifically to the same epitope as the reference antigen binding proteins and antigen binding proteins binding specifically to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen binding protein for steric hindrance to occur.

Usually, when a competing antigen binding protein is present in excess, it will inhibit (e.g., reduce) specific binding of a reference antigen binding protein to a common antigen by at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70% or at least 75% or more.

In some instances, binding is inhibited by at least 80%, at least 85%, at least 90%, at least 95%, or at least 97% or more.

In one embodiment binding is inhibited by at least 95%.

The term "epitope" is a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

The therapeutic antibodies and antigen-binding fragments thereof of the present invention bind preferably to the RBD-domain of the S1-subunit of the SARS-CoV-2 glycoprotein S. In preferred embodiments the therapeutic antibodies and antigen-binding fragments thereof of the present invention bind both to the RBD-domain as well as the S1-subunit of the SARS-CoV-2 glycoprotein S.

The reference antibodies which are selected from a group of amino acids depicted in SEQ IDs NO: 906, 908, 926, 928, 1066, 1068, 1166, 1168, or any combination and/or antigen-binding fragment thereof, define a "sweet-spot" for binding specifically to the RDB and/or S1-subunit of the SARS-CoV-2 glycoprotein S.

In one embodiment the antibodies YU505-A01 (SEQ ID NOs: 889 – 908), YU505-A02 (SEQ ID NOs: 909 - 928), YU505-E01 (SEQ ID NOs: 1049 – 1068) and YU505-F05 (SEQ ID NOs: 1149 - 1168) have been selected as reference antibodies.

In a further embodiment the antibodies YU534-C09 (SEQ ID Nos: 149 – 168), YU534-C12 (SEQ ID NOs: 169 - 188), YU534-D09 (SEQ ID NOs: 509 - 528), YU535-A02 (SEQ ID NOs: 189 - 208), YU536-D04 (SEQ ID NOs: 349 - 368), YU537-H08 (SEQ ID NOs: 769 - 788) and YU537-H11 (SEQ ID NOs: 389 - 408), and STE90-C11 (SEQ ID NO: 1305 - 1312) have been selected as reference antibodies.

In one embodiment the antibody YU505-A02 (SEQ ID NOs: 909 - 928) has been selected as reference antibody. That is, any other antibody or antigen-binding fragment thereof which competes with YU505-A02 for binding specifically to the wild-type RBD of SEQ ID No. 06 and/or for binding specifically to the S1-7PM-mutant (SEQ ID NO. 1730) in a competition assay, is considered a therapeutic antibody or antigen-binding fragment thereof of the present invention.

In other embodiments YU536-D04 (SEQ ID NOs: 349 - 368) is another preferred reference antibody, in yet another embodiment YU537-H11 (SEQ ID NOs: 389 - 408) and/or STE90-C11 (SEQ ID NOs: 1305 - 1312) are preferred reference antibodies.

Thus, in one embodiment the “reference antibody” is selected from YU505-A02 (SEQ ID NOs: 909 - 928), YU536-D04 (SEQ ID NOs: 349 - 368), YU537-H11 (SEQ ID NOs: 389 - 408) and/or STE90-C11 (SEQ ID NOs: 1305 -1312).

The term “sweet spot” as used herein means an epitope of specific preferred quality with respect to the in one embodiment desired characteristics of the neutralizing antibody or antigen-binding fragment thereof. Thus, the binding specifically to the sweet spot is desired in order to achieve therapeutic antibodies with SARS-CoV-2 neutralizing activity.

In one embodiment the “sweet spot” comprises the amino acids 437 to 463 of the SARS-CoV-2 S-protein, which sequence is: 437–NSNNLDSKVGGNYNYLYRLFRKSNLKP–463 (SEQ ID NO: 1811).

Therefore, in one embodiment a therapeutic antibody and/or antigen binding fragment, competes with ACE2 and/or one of the reference antibodies, and/or antigen binding fragment thereof, for binding with at least to one of amino acid to one of the epitopes selected from the group comprising Serine 438, Asparagine 439, Leucine 441, Serine 443, Lysine 444, Valine 445, Glycine 446, Leucine 452, Leucine 455, Phenylalanine 456, Lysine 458, Serine 459, Asparagine 460 and Lysine 462; or any combination thereof, is preferred by this invention.

In yet another embodiment a therapeutic antibody and/or antigen binding fragment thereof, which competes with ACE2 and/or one of the reference antibodies, and/or antigen binding fragment thereof, for binding with at least to one of amino acid to one of the epitopes selected from the group comprising 445-VGGNYNY-451 (SEQ ID NO: 1812), 445- VGGNYNYLYRL-455 (SEQ ID NO: 1813), 445-VGGNYNYLYRLFRKS-459 (SEQ ID NO: 1814) and 449- YNYLYRLFRKS-459 (SEQ ID NO: 1815), or any combination thereof, is more preferred. In yet another embodiment a therapeutic antibody and/or antigen binding fragment, which competes with ACE2 and/or one of the reference antibodies, and/or antigen binding fragment thereof, for binding with at least to one of amino acid of the epitope 445-VGGNYNY-451 is most preferred.

In other embodiments a therapeutic antibody, and/or antigen binding fragment thereof is preferred, which does not bind to the “sweet spot”, but competes with ACE2 and/or one of the reference antibodies, and/or antigen binding fragment thereof, for binding with at least to one of amino acid to one of the epitopes selected from the group comprising 342-VFNTRFASVY-351 (SEQ ID NO: 1816), 349-SVYAWNRRKRIS-359 (SEQ ID NO: 1817), 485-GFNCYFP-491 (SEQ ID NO: 1818) and 497-FQPTNV-502 (SEQ ID NO: 1819), or any combination thereof.

Properties of the therapeutic antibodies and/or fragments thereof according to the present invention

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition with 5 µg/ml IgG of between 24.0% and 102.2%. In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a virus neutralization at 1 µg/ml IgG of between 96% and 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of between 7.75 and 8.31. In yet another embodiment the therapeutic antibody specifically binds with a K_D of between 1×10^{-8} and 1×10^{-10} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of between 93.6 and 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of between 95.9 and 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of between 3.41 and 6.02. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of between 0.5 and 3.14. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of between -0.48 and -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m \text{ onset}}$ of between 57.1 °C and 65.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of between 60.3 °C and 70.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of between 68.0 °C and 81.9 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of between 68.4 °C and 81.2 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of between 24.0% and 102.2% and a virus neutralization at 1 µg/ml IgG of between 96% and 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of between 7.75 and 8.31, and specifically binds with a K_D of between 1×10^{-8} and 1×10^{-10} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of between 93.6 and 100% and a germinality index VL of between 95.9 and 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of between 3.41

and 6.02, a CDR hydrophobicity score of between 0.5 and 3.14 and a CDR total charge of between -0.48 and -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of between 57.1 °C and 65.3 °C and a T_{m1} of between 60.3 °C and 70.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of between 68.0 °C and 81.9 °C and a T_{agg} of between 68.4 °C and 81.2 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC and has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of between 24.0% and 102.2%, a virus neutralization at 1 µg/ml IgG of between 96% and 100%, and an isoelectric point (pI) of human IgG of between 7.75 and 8.31, and specifically binds with a K_D of between 1×10^{-8} and 1×10^{-10} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of between 93.6 and 100%, a germinality index VL of between 95.9 and 100%, a total hydrophobicity score of between 3.41 and 6.02, a CDR hydrophobicity score of between 0.5 and 3.14, and a CDR total charge of between -0.48 and -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of between 57.1 °C and 65.3 °C, a T_{m1} of between 60.3 °C and 70.3 °C, a T_{m2} of between 68.0 °C and 81.9 °C, and a T_{agg} of between 68.4 °C and 81.2 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of between 24.0% and 102.2%, a virus neutralization at 1 µg/ml IgG of between 96% and 100%, an isoelectric point (pI) of human IgG of between 7.75 and 8.31, specifically binds with a K_D of between 1×10^{-8} and 1×10^{-10} to an epitope within the RBD and/or S1-subunit, has a germinality index VH of between 93.6 and 100%, a germinality index VL of between 95.9 and 100%, a total hydrophobicity score of between 3.41 and 6.02, a CDR hydrophobicity score of between 0.5 and 3.14, a CDR total charge of between -0.48 and -0.85, a $T_{m\text{ onset}}$ of between 57.1 °C and 65.3 °C, a T_{m1} of between 60.3 °C and 70.3 °C, a T_{m2} of between 68.0 °C and 81.9 °C, and a T_{agg} of between 68.4 °C and 81.2 °C, shows a „contamination ratio” of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

Properties of the therapeutic antibodies and/or fragments thereof according to the present invention

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition with 5 µg/ml IgG of about 102.2%. In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a virus neutralization at 1 µg/ml IgG of at least 99%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 8.11. In yet another embodiment the therapeutic antibody specifically binds with a K_D of 3.41×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index V_H of about 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index V_L of about 98%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 3.41. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of about 0.5. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of about -0.58. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 61.6 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of at least 68.8 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 81.9 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of at least 71.3 °C. In yet another embodiment the therapeutic antibody shows no multimer or dimer peak in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 102.2% and a virus neutralization at 1 µg/ml IgG of at least 99%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 8.11 and specifically binds with a K_D of 3.41×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index V_H of about 100% and a germinality index V_L of about 98%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 3.41 and a CDR hydrophobicity score of about 0.5 and a CDR total charge of about -0.58. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 61.6 °C, a T_{m1} of at least 68.8 °C, a T_{m2} of at least 81.9 °C and a T_{agg} of at least 71.3 °C. In yet another embodiment the therapeutic antibody shows no multimer or dimer peak in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 102.2%, a virus neutralization at 1 µg/ml IgG of at least 99% and an isoelectric point (pI) of human IgG of about 8.11, and specifically binds with a K_D of 3.41×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 100%, a germinality index VL of about 98%, a total hydrophobicity score of about 3.41 and a CDR hydrophobicity score of about 0.5 and a CDR total charge of about -0.58. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 61.6 °C, a T_{m1} of at least 68.8 °C, a T_{m2} of at least 81.9 °C and a T_{agg} of at least 71.3 °C, and shows no multimer or dimer peak in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 102.2%, a virus neutralization at 1 µg/ml IgG of at least 99%, an isoelectric point (pI) of human IgG of about 8.11, specifically binds with a K_D of 3.41×10^{-9} to an epitope within the RBD and/or S1-subunit, has a germinality index VH of about 100%, a germinality index VL of about 98%, a total hydrophobicity score of about 3.41, a CDR hydrophobicity score of about 0.5, a CDR total charge of about -0.58, a $T_{m\text{ onset}}$ of at least 61.6 °C, a T_{m1} of at least 68.8 °C, a T_{m2} of at least 81.9 °C and a T_{agg} of at least 71.3 °C, shows no multimer or dimer peak in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition with 5 µg/ml IgG of about 43.2%. In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a virus neutralization at 1 µg/ml IgG of at least 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 7.75. In yet another embodiment the therapeutic antibody specifically binds with a K_D of 1.07×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 99.1%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of about 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 6.02. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of about 3.14. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of about -0.63. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 60.1 °C. In yet another embodiment the therapeutic antibody

and/or antigen-binding fragment thereof has a T_{m1} of at least 70.0 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 79.7 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of at least 78.6 °C. In yet another embodiment the therapeutic antibody shows no multimer or dimer peak in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 43.2% and a virus neutralization at 1 µg/ml IgG of at least 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 7.75 and specifically binds with a K_D of 1.07×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 99.1% and a germinality index VL of about 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 6.02 and a CDR hydrophobicity score of about 3.14 and a CDR total charge of about -0.63. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 60.1 °C and a T_{m1} of at least 70.0 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 79.7 °C and a T_{agg} of at least 78.6 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC and has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 43.2%, a virus neutralization at 1 µg/ml IgG of at least 100%, an isoelectric point (pI) of human IgG of about 7.75 and specifically binds with a K_D of 1.07×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 99.1%, a germinality index VL of about 100%, a total hydrophobicity score of about 6.02, a CDR hydrophobicity score of about 3.14 and a CDR total charge of about -0.63. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 60.1 °C, a T_{m1} of at least 70.0 °C, a T_{m2} of at least 79.7 °C and a T_{agg} of at least 78.6 °C, and shows a „contamination ratio” of less than 0.2 in SEC-HLPC and has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof

has a ACE2:RBD inhibition with 5 µg/ml IgG of about 43.2%, a virus neutralization at 1 µg/ml IgG of at least 100%, an isoelectric point (pI) of human IgG of about 7.75, specifically binds with a K_D of 1.07×10^{-8} to an epitope within the RBD and/or S1-subunit, has a germinality index VH of about 99.1%, a germinality index VL of about 100%, a total hydrophobicity score of about 6.02, a CDR hydrophobicity score of about 3.14 and a CDR total charge of about -0.63, a $T_{m\text{ onset}}$ of at least 60.1 °C, a T_{m1} of at least 70.0 °C, a T_{m2} of at least 79.7 °C, a T_{agg} of at least 78.6 °C, shows a „contamination ratio“ of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition with 5 µg/ml IgG of about 24.0%. In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a virus neutralization at 1 µg/ml IgG of at least 97%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 7.88. In yet another embodiment the therapeutic antibody specifically binds with a K_D of 4.84×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of about 97%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 5.18. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of about 1.86. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of about -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 57.1 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of at least 60.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 68.2 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of at least 68.4 °C. In yet another embodiment the therapeutic antibody shows no multimer or dimer peak in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 24.0% and a virus neutralization at 1 µg/ml IgG of at least 97%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 7.88 and specifi-

cally binds with a K_D of 4.84×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6% and a germinality index VL of about 97%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 5.18, a CDR hydrophobicity score of about 1.86 and a CDR total charge of about -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 57.1 °C and a T_{m1} of at least 60.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 68.2 °C and a T_{agg} of at least 68.4 °C. In yet another embodiment the therapeutic antibody shows no multimer or dimer peak in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 24.0%, a virus neutralization at 1 µg/ml IgG of at least 97%, an isoelectric point (pI) of human IgG of about 7.88 and specifically binds with a K_D of 4.84×10^{-9} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6%, a germinality index VL of about 97%, a total hydrophobicity score of about 5.18, a CDR hydrophobicity score of about 1.86 and a CDR total charge of about -0.85. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 57.1 °C, a T_{m1} of at least 60.3 °C, a T_{m2} of at least 68.2 °C and a T_{agg} of at least 68.4 °C, and shows no multimer or dimer peak in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 24.0%, a virus neutralization at 1 µg/ml IgG of at least 97%, an isoelectric point (pI) of human IgG of about 7.88, specifically binds with a K_D of 4.84×10^{-9} to an epitope within the RBD and/or S1-subunit, has a germinality index VH of about 93.6%, a germinality index VL of about 97%, a total hydrophobicity score of about 5.18, a CDR hydrophobicity score of about 1.86, a CDR total charge of about -0.85, a $T_{m\text{ onset}}$ of at least 57.1 °C, a T_{m1} of at least 60.3 °C, a T_{m2} of at least 68.2 °C, a T_{agg} of at least 68.4 °C, and shows no multimer or dimer peak in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition with 5 µg/ml IgG of about 26.7%. In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a virus neutralization at 1 µg/ml IgG of at least 96%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 8.31. In yet another embodiment the therapeutic antibody specifically binds

with a K_D of 1.31×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of about 95.9%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 4.5. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of about 0.44. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of about -0.48. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 57.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of at least 64.5 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 81.6 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of at least 69.5 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio“ of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 26.7% and a virus neutralization at 1 µg/ml IgG of at least 96%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of about 8.31 and specifically binds with a K_D of 1.31×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6% and a germinality index VL of about 95.9%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of about 4.5, a CDR hydrophobicity score of about 0.44 and a CDR total charge of about -0.48. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of at least 57.3 °C and a T_{m1} of at least 64.5 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of at least 81.6 °C and a T_{agg} of at least 69.5 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio“ of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 26.7%, a virus neutralization at 1 µg/ml IgG of at least 96%, an isoelectric point (pI) of human IgG of about 8.31 and specifically binds

with a K_D of 1.31×10^{-8} to an epitope within the RBD and/or S1-subunit. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of about 93.6%, a germinality index VL of about 95.9%, a total hydrophobicity score of about 4.5, a CDR hydrophobicity score of about 0.44 and a CDR total charge of about -0.48. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m \text{ onset}}$ of at least 57.3 °C, a T_{m1} of at least 64.5 °C, a T_{m2} of at least 81.6 °C and a T_{agg} of at least 69.5 °C, shows a „contamination ratio“ of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition with 5 µg/ml IgG of about 26.7% and a virus neutralization at 1 µg/ml IgG of at least 96%, has an isoelectric point (pI) of human IgG of about 8.31, specifically binds with a K_D of 1.31×10^{-8} to an epitope within the RBD and/or S1-subunit. has a germinality index VH of about 93.6%, a germinality index VL of about 95.9%, a total hydrophobicity score of about 4.5, a CDR hydrophobicity score of about 0.44, a CDR total charge of about -0.48, a $T_{m \text{ onset}}$ of at least 57.3 °C, a T_{m1} of at least 64.5 °C, a T_{m2} of at least 81.6 °C, a T_{agg} of at least 69.5 °C and shows a „contamination ratio“ of less than 0.2 in SEC-HLPC, has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibodies show a $T_{m \text{ onset}}$ above 60°C, more preferred above 65°C, most preferred above 70°C. In another embodiment the therapeutic antibodies show a T_{m1} above 60°C, more preferred above 65°C, most preferred above 70°C. In another embodiment the therapeutic antibodies show a T_{m2} above 70°C, more preferred above 75°C, most preferred above 80°C. In another embodiment the therapeutic antibodies show a T_{agg} above 70°C, more preferred above 75°C, most preferred above 80°C.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition of the scFV of 87%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of 8.11. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 96.3%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of 99%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 4.64. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of 1.29. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 0.82. In yet another embodiment the therapeutic antibody

and/or antigen-binding fragment thereof has a CDR total charge of -0.19. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\ onset}$ of about 63.7 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of about 70.8 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of about 71.7 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFV of 87% and has an isoelectric point (pI) of human IgG of 8.11. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 96.3% and a germinality index VL of 99%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 4.64 and a CDR hydrophobicity score of 1.29. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 0.82 and a CDR total charge of -0.19. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\ onset}$ of about 63.7 °C, a T_{m1} of about 70.8 °C and a T_{agg} of about 71.7 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC and no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFV of 87%, an isoelectric point (pI) of human IgG of 8.11, a germinality index VH of 96.3% and a germinality index VL of 99%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 4.64, a CDR hydrophobicity score of 1.29, an scFv charge of 0.82 and a CDR total charge of -0.19. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\ onset}$ of about 63.7 °C, a T_{m1} of about 70.8 °C and a T_{agg} of about 71.7 °C, shows a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFV of 87%, an isoelectric point (pI) of human IgG of 8.11, a germinality index VH of 96.3%, a germinality index VL of 99%, a total hydrophobicity score of 4.64, a CDR hydrophobicity score of 1.29, an scFv charge of 0.82, a CDR total charge of -0.19, a T_m

onset of about 63.7 °C, a T_{m1} of about 70.8 °C, a T_{agg} of about 71.7 °C, shows a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition of the scFv of 27%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of 8.11. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 84.4.3%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 5.08. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of 2.09. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 0.18. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of -0.83. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFv of 27% and an isoelectric point (pI) of human IgG of 8.11. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 84.4.3% and a germinality index VL of 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 5.08 and a CDR hydrophobicity score of 2.09. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 0.18 and a CDR total charge of -0.83. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC and no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFv of 27%, an isoelectric point (pI) of human IgG of 8.11, a germinality index VH of 84.4.3% and a germinality index VL of 100%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 5.08, a CDR hydrophobicity score of 2.09, an scFv charge of 0.18 and a CDR total charge of -0.83. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce

ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFV of 27%, an isoelectric point (pI) of human IgG of 8.11, a germinality index VH of 84.4.3%, a germinality index VL of 100%, a total hydrophobicity score of 5.08, a CDR hydrophobicity score of 2.09, an scFv charge of 0.18, a CDR total charge of -0.83, shows a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody has a ACE2:RBD inhibition of the scFV of 49%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an isoelectric point (pI) of human IgG of 8.39. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 95.4%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VL of 96.9%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 4.37. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR hydrophobicity score of 1.45. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 1.22. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a CDR total charge of -0.25. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\ onset}$ of about 62.4 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m1} of about 69.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of about 81.4 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{agg} of about 69.9 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations. In yet another embodiment the therapeutic antibody does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFV of 49% and an isoelectric point (pI) of human IgG of 8.39. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a germinality index VH of 95.4% and a germinality index VL of 96.9%. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a total hydrophobicity score of 4.37 and a CDR hydrophobicity score of 1.45. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has an scFv charge of 1.22 and a CDR total charge of -0.25. In yet another embodiment the therapeutic antibody and/or antigen-binding

fragment thereof has a $T_{m\text{ onset}}$ of about 62.4 °C and a T_{m1} of about 69.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of about 81.4 °C and a T_{agg} of about 69.9 °C. In yet another embodiment the therapeutic antibody shows a „contamination ratio” of less than 0.2 in SEC-HLPC. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFv of 49%, an isoelectric point (pI) of human IgG of 8.39, a germinality index VL of 96.9%, a total hydrophobicity score of 4.37, a CDR hydrophobicity score of 1.45, an scFv charge of 1.22, a CDR total charge of -0.25, a $T_{m\text{ onset}}$ of about 62.4 °C and a T_{m1} of about 69.3 °C. In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a T_{m2} of about 81.4 °C, a T_{agg} of about 69.9 °C, a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a ACE2:RBD inhibition of the scFv of 49%, an isoelectric point (pI) of human IgG of 8.39, a germinality index VL of 96.9%, a total hydrophobicity score of 4.37, a CDR hydrophobicity score of 1.45, an scFv charge of 1.22, a CDR total charge of -0.25, a $T_{m\text{ onset}}$ of about 62.4 °C, a T_{m1} of about 69.3 °C, a T_{m2} of about 81.4 °C, a T_{agg} of about 69.9 °C, a „contamination ratio” of less than 0.2 in SEC-HLPC, no unfavourable cysteines and/or glycosylations and does not induce ADE or cytokine storm syndrome within a patient.

In one embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 61.6 ± 0.1 °C and/or a T_{m1} of 68.8 ± 0.1 °C, and/or a T_{m2} of 81.9 ± 0.1 °C, and/or a T_{agg} of 71.3 ± 0.1 °C.

In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 60.1 ± 0.2 °C and/or a T_{m1} of 70.0 ± 0.2 °C, and/or a T_{m2} of 79.7 ± 0.1 °C, and/or a T_{agg} of 78.6 ± 0.2 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 57.1 ± 0.3 °C and/or a T_{m1} of 60.3 ± 0.0 °C, and/or a T_{m2} of 68.2 ± 0.1 °C, and/or a T_{agg} of 68.4 ± 0.1 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 57.3 ± 0.3 °C and/or a T_{m1} of 64.5 ± 0.0 °C, and/or a T_{m2} of 81.6 ± 0.1 °C, and/or a T_{agg} of 69.5 ± 0.3 °C.

In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 65.3 ± 0.8 °C and/or a T_{m1} of 69.5 ± 0.0 °C, and/or a T_{m2} of 81.4 ± 0.7 °C, and/or a T_{agg} of 71.2 ± 0.0 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 62.4 ± 0.1 °C and/or a T_{m1} of 68.9 ± 0.0 °C, and/or a T_{m2} of 81.4 ± 0.1 °C, and/or a T_{agg} of 69.9 ± 0.0 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 62.2 ± 0.1 °C and/or a T_{m1} of 70.8 ± 0.2 °C, and/or a T_{m2} of 81.9 ± 0.2 °C, and/or a T_{agg} of 75.9 ± 0.0 °C.

In another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 62.4 ± 0.0 °C and/or a T_{m1} of 69.3 ± 0.1 °C, and/or a T_{m2} of 80.9 ± 0.0 °C, and/or a T_{agg} of 81.2 ± 0.3 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 56.9 ± 0.2 °C and/or a T_{m1} of 62.1 ± 0.2 °C, and/or a T_{m2} of 68.0 ± 0.2 °C, and/or a T_{agg} of 70.1 ± 0.1 °C.

In yet another embodiment the therapeutic antibody and/or antigen-binding fragment thereof has a $T_{m\text{ onset}}$ of 63.7 ± 0.0 °C and/or a T_{m1} of 70.0 ± 0.2 °C, and/or a T_{agg} of 71.7 ± 0.2 °C. A further aspect, the present invention relates to a combination comprising at least 2, 3, 4, 5, 6 or more of the above monoclonal antibodies, particularly monoclonal antibodies or antigen-binding fragments thereof. In such a combination, the individual antibodies or fragments are present in suitable molar ratios. Typically, the molar ratios are in the range of about 1:5 to 5:1, particularly about 1:2 to 2:1. In certain embodiments, the antibody combination may comprise a single composition wherein the antibodies and antibody fragments in said composition consist of a predetermined number of different species of monoclonal antibodies or antibody fragments as described above.

The antibody combination may comprise a plurality of compositions each comprising a different species of monoclonal antibody or antibody fragment as described above. The combination of the present invention may be free from other antibodies or antibody fragments, particularly from polyclonal antibodies or antibody fragments.

In certain embodiments, the combination of antibodies or antigen-binding fragments has an IC_{50} value of about 20 nM or less, of about 10 nM or less, or of about 5 nM or less, particularly when determined in molar ratios of 1:1 of the individual antibodies or antibody fragments as described in the present examples.

In certain embodiments, the combination of antibodies or antigen-binding fragments has an EC_{50} value of about 20 nM or less, of about 10 nM or less, or of about 5 nM or less, particularly when determined in molar ratios of 1:1 of the individual antibodies or antibody fragments as described in the present examples.

In particular preferred are combinations of at least two antibodies selected from the group consisting of antibodies STE90-C11, YU505-A02, YU536-D04 and YU537-H11.

In a further embodiment, the combination is selected from the group consisting of antibodies YU534-C09 and YU534-C12, YU534-C09 and YU534-D09, YU534-C09 and YU535-A02, YU534-C09 and YU536-D04, YU534-C09 and YU537-H08, YU534-C09 and YU537-H11, YU534-C09 and STE90-C11, YU534-C12 and YU534-D09, YU534-C12 and YU535-A02, YU534-C12 and YU536-D04, YU534-C12 and YU537-H08, YU534-C12 and YU537-H11, YU534-C12 and STE90-C11, YU534-D09 and YU535-A02, YU534-D09 and YU536-D04, YU534-D09 and YU537-H08, YU534-D09 and YU537-H11, YU534-D09 and STE90-C11, YU536-D04 and YU537-H08, YU536-D04 and YU537-H11, YU536-D04 and STE90-C11, YU537-H08 and YU537-H11, YU537-H08 and STE90-C11, YU537-H11 and STE90-C11.

In a further embodiment, the combination is selected from the group consisting of antibodies STE70-1E12 and STE72-8E1, STE70-1E12 and STE73-9G3, STE70-1E12 and STE73-2G8, STE72-8A6 and STE73-9G3, STE72-8A6 and STE73-2E9, STE72-8E1 and STE73-2B2, STE72-8E1 and STE72-8A2, STE73-9G3 and STE73-2B2, STE73-9G3 and STE72-8A2, STE73-2B2 and STE73-2C2, STE73-2B2 and STE73-2E9, STE73-2B2 and STE73-2G8, STE72-8E1 and STE72-2G4, STE72-8E1 and STE73-6C8, STE73-2B2 and STE73-6C1, STE73-2C2 and STE73-6C8, STE73-2E9 and STE72-2G4, STE73-2G8 and STE73-6C8, STE73-6C1 and STE72-2G4, STE73-6C1 and STE73-6C8, STE72-4C10 and STE73-2B2, STE72-4C10 and STE72-8A2 and STE72-4C10 and STE72-8E1.

The CDR, VH and/or VL sequences of the corresponding antibodies can be taken from the sequence listing and/or tables 1, 4-7.

The term "antigen" refers to a molecule or a portion of a molecule capable of being bound by a selective binding agent, such as an antigen binding protein (including, e.g., an antibody or immunological functional fragment thereof). In some embodiments, the antigen is capable of being used in an animal to produce antibodies capable of binding specifically to that antigen. An antigen can possess one or more epitopes that are capable of interacting with different antigen binding proteins, e.g., antibodies.

In specific embodiments, the antibody or antigen binding fragment thereof for use in the method of the invention may be monospecific, bispecific, or multispecific. Multispecific antibodies may

be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide.

The specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope.

Moreover, multi-specific antibodies (e.g., bispecifics) that bind to S1-protein and/or RBD and one or more additional antigens are nonetheless considered antibodies that "specifically bind" S1-protein or RBD, as used herein.

The term "therapeutically effective amount" means an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

In another embodiment, the invention pertains to a method for treating COVID-19 which is ameliorated, improved, inhibited or prevented with the antibodies and antigen-binding fragments therein, comprising administering a therapeutic amount of the pharmaceutical composition to a subject in need thereof.

In yet another embodiment, the invention pertains to an antibody or antigen-binding fragment thereof for use to attenuate or inhibit a SARS-CoV-2 mediated disease or condition.

In yet another embodiment, the invention pertains to the use of an antibody or antigen-binding fragment thereof in the manufacture of a medicament for use to attenuate or inhibit a SARS-CoV-2-mediated disease or condition.

In yet another embodiment, the invention pertains to one of the uses mentioned herein, wherein the SARS-CoV-2-mediated disease or condition is pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD) with diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC), leukoerythroblastic reaction and microvesicular steatosis in the liver.

In yet another embodiment, the invention pertains to the diagnostic use the antibody or antigen-binding fragment thereof. Uses include but are not limited to the detection of infections, recognition of allergies and the measurement of viral load and/or other biological markers in blood.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described.

Preparation of Human Antibodies

A fully human monoclonal antibody or antigen-binding fragment thereof can be produced in three ways.

In one approach, a mouse is used in which the immunoglobulin genes have been replaced with human genes. This mouse then makes antibody responses that use human antibody sequences.

A second approach uses a bacteriophage display library of human variable region sequences and selects for antigen binding in vitro. The selected genes are then combined with human constant region genes to reconstitute a complete IgG antibody.

A third approach utilizes B-cells for the production of antibodies.

As will be outlined in the examples in more detail, suspension panning may be used for high throughput antibody identification.

Notwithstanding, these human monoclonal antibody or antigen-binding fragment thereof can provoke immune responses (termed HAHA, or human anti-human antibody), probably largely akin to anti-idiotypic responses that are themselves specific for the reagents' complementarity determining regions.

Production of “antigens” and reference-proteins

The antigens such as SARS-CoV-2 glycoprotein S and reference-proteins such as (human) ACE2 may be produced via isolation from an infected cell or host, or via conventional biotechnological methods, such as recombinant expression of the protein in a host cell, such as *E. coli*, yeast or the like.

Also fragments may be used according to this invention. As such the term “fragments” comprises preferably “functional fragments” which means parts of the full-length protein, which retains its specific function, such as for example binding activity, activation activity, inhibition activity and/or enzymatic activity. Normally such a functional fragment is a subunit and/or a domain of a protein, which is correctly three-dimensional folded when recombinantly expressed; or it is

produced by isolating or recombinantly expressing the correctly folded full-length protein and then treating the full-length protein with a protease or other enzymatic or physical treatment which disconnects the subunit and/or domain of interest from the rest of the protein, thereby retaining its biological function.

Preferred functional fragments of the present invention include the receptor binding domain of the SARS-CoV-2 glycoprotein S, the S1 subunit of the SARS-CoV-2 glycoprotein S, the extra-cellular part of (human) ACE2, S1-mFc, S1-S2-His, RBD-hFc, IVIGs (intravenous immunoglobulins), as well as any fragment of an antibody which retains its binding ability to the antigen, etc.

K_D-Measurement

As will be outlined in more detail in the examples, the K_D is measured for example with a protein A sensor loaded with the antibody or antigen-binding fragment thereof.

The antigen, for example a S1-HIS-fragment derived from the S1-subunit according SEQ-ID NO: 04, or a RBD of SEQ ID NO: 06, or the full S-protein of SARS-CoV-2 of SEQ-ID NO: 02 is then associated onto the antibody-loaded sensor. In this step the antigen is bound in different dilutions to the antibody-loaded sensor. Preferred dilutions range from 0 to 500 nM, in one embodiment a concentration regime of 0 nM, 7.8 nM, 15.6 nM, 31.2 nM, 62.5 nM, 125 nM, 250 nM and 500 nM antigen diluted in 1% BSA-PBST (0.05% Tween 20).

Then each antibody-antigen loaded sensor is incubated with dissociation buffer and the dissociation is measured.

This measurement may be repeated after regeneration of the sensor by incubation with glycine in order to remove the antibody from the protein A and neutralization by incubation in PBS to neutralize the pH. Normally the measurement is repeated at least 5 times, 10 times, 15 times to achieve reliable K_D-values.

The sensor-material (protein A) without any antibody serves as a negative control in these measurements.

Further, the present invention relates to a nucleic acid molecule, e.g. a DNA molecule, encoding an antibody VH region, or an antibody VL region, or encoding a complete antibody or an antibody fragment as indicated above, a vector or vector system, i.e. a plurality of vectors, comprising said nucleic acid molecule(s) as indicated above in operative linkage with an expression control sequence, particularly with a heterologous expression control sequence. Furthermore, the invention relates to a cell comprising a nucleic acid molecule or a vector or vector system as described above. Vectors for the recombinant production of antibodies are well known in the art.

The cell may be known host cell for producing antibodies or antibody fragments, e.g. a prokaryotic cell such as an E. coli cell, a yeast cell, an insect cell or a mammalian cell, e.g. a CHO cell or a hybridoma cell.

Still a further aspect of the present invention is a method of recombinantly producing an antibody or antibody fragment by growing a cell as described above in a culture medium and obtaining the antibody or antibody fragment from the cell or the culture medium. Suitable culture media and culture conditions are well known in the art.

Binding of therapeutic antibodies to SARS-CoV-2-“escape”-mutants

In one embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof, which does not only bind to the glycoprotein S with the amino acid sequence of SEQ ID NO: 02 and neutralizes the infectious activity of SARS-CoV-2, but which also shows specific binding specifically to the “escape”-mutants of the glycoprotein S of SARS-CoV-2 in order to neutralize the infectious activity of these SARS-CoV-2-mutants. This is in contrast to prior-art anti-SARS-CoV-2 antibodies, whose binding is blocked by known RBD mutations, endowing the antibody of the present invention with intrinsic higher resistance to possible escape mutants.

The “escape”-mutation mentioned herein is selected from the list consisting of S1-V367F, S1-N439K, S1-G476S, S1-V483A, S1-E484K, S1-G485R, S1-F486V and S1-7PM (SEQ ID NO: 1730).

The mutations were published in the following sources:

- V367F: GISAID EpiFlu™ Database
- N439K: GISAID EpiFlu™ Database
- G476S: Shi et al. (<https://doi.org/10.1038/s41586-020-2381-y>)
- V483A: Shi et al.
- E484K: Baum et al. (<https://doi.org/10.1126/science.abd0831>)
- G485R: GISAID EpiFlu™ Database
- F486V: Baum et al.

All of these mutations lie in the three regions identified by Yi et al. (Cell. & Mol. Imm., 15 May 2020) which are in the regions of between aa 439 to 456; aa 473 to 476 and aa 483 to 505.

In one aspect the present invention discloses even a therapeutic antibody or antigen-binding fragment thereof, which binds specifically to the S1-7PM-mutant (SEQ ID NO: 1730), i.e. a mutant which comprises all seven "escape"-mutations in one molecule.

Thus, one advantage of the inventive antibodies is the recognition of a large number of known "escape"-mutations. This allows the treatment of a wide range of patients with just one antibody-composition, simplifying the administration scheme and lowering costs for production and market-approval significantly. This is advantageous especially in comparison to compositions comprising cocktails of two or more monoclonal antibodies.

Furthermore, without being bound to theory, it seems that the inventive antibodies recognize a "sweet spot" in the glycoprotein S, which seems to be important for the interaction of the virus with the host cell and, thus, cannot be changed without loss of infectivity or viral function. Thus, another technical advantage of the inventive antibodies is that the likelihood of the virus mutating in a way which "escapes" the antibody is significantly reduced.

Some experiments were conducted with a number of antibodies against the seven "escape"-mutants. As control the two published antibodies RGN10933 and RGN10987 were used.

ELISA titrating Abs on wild-type and mutated S1-protein

It turned out that all antibodies produced by the selected method showed a good binding profile to all "escape"-mutants including the S1-7PM-mutant (cf. example 11 and figures 29 - 31). In contrast, RGN10933 did not bind to the S1-7PM-mutant and showed reduced binding specifically to at least the F486V-mutant.

An affinity test of the selected Abs against mutated S1-protein

The overall affinity of the inventive antibodies against the targets was tested in a BLI-assay (cf. example 13 and figures 33). The affinity was still high enough to qualify the antibodies as therapeutic antibodies, although reduced against the "escape"-mutants as compared to the "wild-type"-S1-protein.

Again, RGN10933 showed a significant weaker binding specifically to some mutants, and the binding specifically to F486V and S1-7PM was so low that it could not be measured.

Affinity tests of the selected Abs against further RBD-mutants

Recently, several SARS-CoV-2 variants with mutations in the RBD emerged. Here most prominent are the B.1.1.7 ("UK", RBD mutation N501Y), B.1.351 ("Southafrica", K417N, E484K, N501Y) and P1 (B.1.1.28.1) ("Brazil", K417T, E484K, N501Y) (Rees-Spear et al., 2021). More

recently other variants like B.1.429+B.1.427 ("Southern California", L452R) (Zhang et al., 2021), B.1.526 ("New York", E484K, in some variants S477N instead of E484K) (Annavajhala et al., 2021), B.1.258Δ ("Czech", N439K) (Brejová et al., 2021; Surleac et al., 2021), P2 (B.1.1.28.2) (E484K) (Nonaka et al., 2021), P3 (B.1.1.28.3) (E484K, N501Y) (Tablizo et al., 2021), B.1.1.33 (E484K) (Resende et al., 2021), B.1.617 ("India", L452R, E484Q) (Ranjan et al., 2021) and other variants like B.1.525 (E484K) (Hodcroft et al., 2021) occurred.

YU537-H11, YU536-D04 and STE90-C11 were tested against a number of RBD-mutants selected from the list comprising F486V-mutant, G485D-mutant, E484K-mutant, F486A-mutant, K417N/T-mutant, B.1.1.7 (N501Y)-mutant, B.1.351 (K417N, E484K, N501Y)-mutant, B.1.1.28.1 (K417T, E484K, N501Y)-mutant, B.1.429/B.1.427 (L452R) -mutant, B.1.526 (E484K or S477N)-mutant, B1.258Δ (N439K)-mutant, B.1.525 (E484K)-mutant, B.1.1.28.2 (E484K)-mutant, B.1.617 (L452R, E484Q)-mutant and B.1.1.33 (E484K)-mutant.

It turned out that YU537-H11 (SEQ ID. No.: 389-408), YU536-D04 (SEQ ID No.: 349-368) and STE90-C11 (SEQ ID No.: 1305 – 1312) were still binding to most analyzed RDB mutants (see examples 15-18).

Thus, in one embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof which binds to at least one RBD-mutant selected from F486V-mutant, G485D-mutant, E484K-mutant, F486A-mutant, K417N/T-mutant, N501Y-mutant, K417N / E484K / N501Y-triple-mutant, K417T / E484K, / N501Y-triple-mutant, L452R-mutant, E484K and/or S477N-mutant, N439K-mutant, and L452R / E484Q-double-mutant.

Thus, in one embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof, which binds to at least one of said mutants, preferably to at least 2 of said mutants, more preferably to at least 3 of said mutants; more preferably to at least 4 of said mutants; more preferably to at least 5 of said mutants; and up to 8 of said mutants, more preferably up to 9 of said mutants, even more preferably up to 10 of said mutants, and most preferably up to all of them.

Without being bound to theory, it is likely that the wide interface area and the extensive light chain contacts may compensate the exchange of several amino acids in the target sequence. The calculated interface area of STE90-C11 VL is 1133 Å², and, thus, more than twice the area of REGN10933 and REGN10987 and slightly larger compared to CB6 or CR3022.

Thus, in one embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof, which does have an interface area with the RBD of at least 800 Å², more preferred of at least 900 Å², even more preferred of at least 1000 Å²; even more preferred of at least 1100 Å², most preferred of at least 1130 Å² and more, as calculated with the PISA-tool as

published in E. Krissinel and K. Henrick (2007). 'Inference of macromolecular assemblies from crystalline state.'. J. Mol. Biol. 372, 774—797 and further explained in E. Krissinel and K. Henrick (2005). Detection of Protein Assemblies in Crystals. In: M.R. Berthold et.al. (Eds.): CompLife 2005, LNBI 3695, pp. 163—174. Additional details of the algorithm is disclosed in E. Krissinel (2009). Crystal contacts as nature's docking solutions. J Comput Chem. 2010 Jan 15;31(1):133-43.; DOI 10.1002/jcc.21303.

In one embodiment said antibody is selected from the list comprising YU537-H11 (SEQ ID. No.: 389-408), YU536-D04 (SEQ ID. No.: 349 – 368) and STE90-C11 (SEQ ID. No.: 1305 – 1312), and/or any combination thereof.

Conserved CDR-Sequences

A unifying sequence concept was developed confirming the earlier finding that the antibodies of the present invention represent a cluster of antibodies which shows advantageous features when it comes to the ability to recognize SARS-CoV-2-mutants.

In detail the sequences of the light and heavy chains of STE90-C11 (SEQ ID NO.: 1305 & 1309), YU536-D04 (SEQ ID NO.: 362 & 364) and YU537-H11 (SEQ ID NO.: 402 & 404) were aligned with the software tool Clustal™-Omega. As it comes apparent from the amino acid sequence alignment, in at least one embodiment conserved amino acids could be identified in the CDR-regions which apparently are of importance when it comes to the advantageous binding abilities of the antibodies of the present invention (cf. also example 16, figures 43 & 44) and which supports the finding that all inventive antibodies are structural related to each other.

Thus, “archetype” CDR-sequences can be identified, which are preferred:

In one embodiment the light-chain CDR1 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to RASQGISSYLA (SEQ ID No. 1831); and/or the CDR2 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to AASTLQS (SEQ ID No. 1832) and/or the CDR3 is, except a maximum of three amino acids, preferably except a maximum of two amino acids, most preferably except a maximum of only one amino acid, identical to QQLNSYP(P/-)(F/I)T (SEQ ID No. 1833) (cf. also Figure 43).

Wherein “-” represents a deletion of an amino acid (in this case the deletion of one P); (F/I) or the like represents a conservative amino acid substitution between for example phenylalanine and isoleucine.

In one embodiment the heavy-chain CDR1 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to SNYMS (SEQ ID No. 1834); and/or the

CDR2 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to VIYSGGSTYYADSVKG (SEQ ID No. 1835) and/or the CDR3 is represented by the general sequence D(V/L)(a/v)XX(a/g)(F/L)D(I/V) (SEQ ID No. 1836) (cf. also Figure 43). Wherein X represents any natural occurring amino acid; (V/L) or the like represents a conservative amino acid substitution between for example valine and leucine; and wherein (a/v) or the like represents a semi-conservative amino acid substitution between for example alanine and valine.

In another embodiment the light-chain CDR1 is represented by the generalized sequence X.S..XXX..Y:: (SEQ ID No. 1837) wherein X represents any natural occurring amino acid (or in positions 6 and 7 a deletion); a period "." represents a semi-conservative amino acid exchange for example as follows: in position 2 preferably a glycine (G) or alanine (A); in position 4 preferably a serine (S) or glutamine (Q); in position 5 preferably a serine (S) or glycine (G); in position 9 preferably an asparagine (N) or serine (S); in position 10 preferably an asparagine (N) or serine (S); and wherein a colon ":" represents a conservative amino acid exchange for example as follows: in position 12 preferably a valine (V) or leucine (L); in position 13 preferably a serine (S) or alanine (A). In another embodiment the light-chain CDR1 comprises at least in position 3 a serine and in position 11 a tyrosine; i.e. as depicted in the following generalized sequence XXSXXXXXXXXYXX (SEQ ID No. 1838) wherein X represents any natural occurring amino acid (cf. also Figure 44).

In another embodiment the light-chain CDR2 is represented by the generalized sequence X:..XXS (SEQ ID No. 1839); wherein X represents any natural occurring amino acid; a period "." represents a semi-conservative amino acid exchange for example as follows: in position 3 preferably an asparagine (N) or serine (S); in position 4 preferably a lysine (K) or threonine (T); and wherein a colon ":" represents a conservative amino acid exchange for example as follows: in position 2 preferably a threonine (T) or alanine (A). In another embodiment the light-chain CDR2 comprises at least in position 7 a serine; i.e. as depicted in the following generalized sequence XXXXXXS (SEQ ID No. 1840); wherein X represents any natural occurring amino acid (cf. also Figure 44).

In another embodiment the light-chain CDR3 is represented by the generalized sequence XXX:SXXXXX. (SEQ ID No. 1841); wherein X represents any natural occurring amino acid or deletion; a period "." represents a semi-conservative amino acid exchange for example as follows: in position 11 preferably a valine (V) or threonine (T); and wherein a colon ":" represents a conservative amino acid exchange for example as follows: in position 4 preferably an asparagine (N) or aspartic acid (D). In another embodiment the light-chain CDR3 comprises at least in position 5 a serine; i.e. as depicted in the following generalized sequence XXXXSXXXXX (SEQ ID No. 1842); wherein X represents any natural occurring amino acid or in positions 8

and/or 9 a deletion (cf. also Figure 44).

In another embodiment the heavy-chain CDR1 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to SNYMS (SEQ ID No. 1834); and/or the CDR2 is, except a maximum of two amino acids, preferably except a maximum of only one amino acid, identical to VIYSGGSTYYADSVKG (SEQ ID No. 1835) and/or the CDR3 is represented by the general sequence (G/D)XXXXXX(a/g)(M/F/L)D(I/V) (SEQ ID No. 1843) (cf. also Figure 43); wherein X represents any natural occurring amino acid (and/or in positions 4, 5 and/or 6 a deletion); (M/F/L) or the like represents a conservative amino acid substitution between for example methionine, phenylalanine and leucine; and wherein (a/g) or the like represents a semi-conservative amino acid substitution between for example alanine and glycine. In another embodiment the heavy-chain CDR3 comprises at least in position 10 an aspartic acid; i.e. as depicted in the following generalized sequence XXXXXXXXXDX (SEQ ID No. 1844); wherein X represents any natural occurring amino acid (and/or in positions 4, 5 and/or 6 a deletion) (cf. also Figure 44).

A “conservative amino acid substitution” (also called a conservative mutation or a conservative replacement) is defined herein as an amino acid replacement in a protein that changes a given amino acid to a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size).

There are 20 naturally occurring amino acids, however some of these share similar characteristics. For example, leucine and isoleucine are both aliphatic, branched hydrophobes. Similarly, aspartic acid and glutamic acid are both small, negatively charged residues.

Although there are many ways to classify amino acids, they are often sorted into six main classes on the basis of their structure and the general chemical characteristics of their side chains (R groups).

Class 1 “aliphatic”: glycine, alanine, valine, leucine, isoleucine (G, A, V, L, I); class 2 “hydroxyl or sulfur/selenium-containing”: serine, cysteine, selenocysteine, threonine, methionine (S, C, U, T, M); class 3 “cyclic”: proline (P); class 4 “aromatic”: phenylalanine, tyrosine, tryptophan (F, Y, W); class 5 “basic”: histidine, lysine, arginine (H, K, R); class 6 “acidic and their amides”: aspartate, glutamate, asparagine, glutamine (D, E, N, Q).

Physicochemical distances aim at quantifying the intra-class and inter-class dissimilarity between amino acids based on their measurable properties, and many such measures have been proposed in the literature. Owing to its simplicity, one of the most commonly used measures is the one of Miyata et al (1979). A conservative replacement is therefore an exchange between two amino acids separated by a small physicochemical distance. Conservative replacements in

proteins often have a smaller effect on function than non-conservative replacements.

A conservative exchange may be also defined with the help of the Clustal™ software, preferable with Clustal™-Omega. Therein asterisk “*” is defined as positions that have a single and fully conserved residue; colon “:” is defined as conservation between groups of strongly similar properties with a score greater than 0.5 on the PAM 250 matrix; and period “.” is defined as conservation between groups of weakly similar properties with a score less than or equal to 0.5 on the PAM 250 matrix.

The sequence similarity also supports the inventive idea of a compatibility of the different light and heavy chains with each other. As could be shown in example 19 (cf. figure 46) also hybrid-antibodies with light chain and heavy chain of different sources resulted not only in good binding to the wild-type RBD, but even in improved binding to some critical mutants.

Thus, in one embodiment the inventive antibodies, or antigen-binding fragments thereof, may be combined in their light or heavy chain, in some embodiments in their CDR-domains, in order to achieve further antibodies with the effect of both recognition of many RBD-mutants as well as excellent neutralizing activity.

Animal tests with the inventive antibodies

The inventive antibodies, or antigen-binding fragments thereof, show also good reduction of viral load in animals, such as hamsters or mice (cf. example 17).

Thus, in one embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof, which in one embodiment reduces the viral titer in vivo from 10^5 or more to 10^3 or less after 3 days of treatment and/or from 10^5 or more to 10^2 or less after 5 days of treatment. In yet another embodiment the therapeutic antibody or antigen-binding fragment thereof reduces the viral titer in vivo from 10^6 or more to 10^4 or less after 3 days of treatment and/or from 10^6 or more to 10^3 or less after 5 days of treatment. In one embodiment said antibody is selected from the list comprising YU537-H11 (SEQ ID. No.: 389-408), YU536-D04 (SEQ ID. No.: 349 – 368) and STE90-C11 (SEQ ID. No.: 1305 – 1312), and/or any combination thereof.

Thus, in yet another embodiment the present invention provides a therapeutic antibody or antigen-binding fragment thereof, which prevents a severe course of the COVID-19 disease upon treatment. In one embodiment said antibody is selected from the list comprising YU537-H11 (SEQ ID. No.: 389-408), YU536-D04 (SEQ ID. No.: 349 – 368) and STE90-C11 (SEQ ID. No.: 1305 – 1312), and/or any combination thereof.

Preventive use of the inventive antibodies

As already described in other parts of this specification, the therapeutic use of the antibodies or antigen-binding fragments thereof of the present invention, may include

- (1) the prevention or reduction of the infectivity of SARS-CoV-2;
- (2) the prevention or reduction of any overreaction of the immune-system of the host (e.g. ADE-reaction or cytokine storm syndrome), and/or
- (3) the prevention or reduction of any condition associated with COVID-19, such as fever, cough, fatigue, shortness of breath, loss of smell and taste, pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD), diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC), leukoerythroblastic reaction, microvesicular steatosis in the liver and any combination thereof.

In one aspect of the present invention the antibodies or antigen-binding fragments thereof of the present invention are applied if the patient shows at least one of the following symptoms: manifested, productive and unproductive cough, fever, cold, disturbance of the olfactory and / or taste sensors, pneumonia, sore throat, shortness of breath, headache and body aches, loss of appetite, weight loss, nausea, abdominal pain, vomiting, diarrhea, conjunctivitis, skin rash, lymph node swelling, apathy, somnolence.

In yet another aspect, the person to be treated with the antibodies or antigen-binding fragments thereof of the present invention may be asymptomatic or pre-symptomatic, but has an elevated risk of developing a COVID-19 disease, because, for example, belonging to a risk group, e.g. with an age above 60 years, above 65 years, above 70 years, above 75 years; and/or because the person having a disease which is known to elevate the risk of a severe COVID-19 disease, such as diabetes, asthma, obesity, cardiovascular diseases, COPD, immune-deficiency, cancer, or the like; and/or because the person was exposed or will be exposed to another person which was tested positive for a COVID-19 disease; and/or because the person has a biomarker (e.g. blood group, IL-6-levels, etc.) , which deviation from the norm (e.g. presence, elevation or decrease as compared to control group) is associated with an elevated risk of a severe COVID-19 disease.

In another aspect of the invention the antibodies or antigen-binding fragments thereof of the

present invention are applied if the patient is pregnant in order to prevent a COVID-19 disease.

In yet another aspect of the invention the antibodies or antigen-binding fragments thereof of the present invention are applied, if the patient is suspected to develop or has already developed a multisystem inflammatory syndrome, which mainly seems to occur in children (MIS-C).

According to the CDC classification, one speaks of a MIS-C if the following criteria are met:

- A person aged <21 years with fever, inflammation in the laboratory AND
- Signs of a clinically serious illness that requires hospitalization with a multisystem (> 2) organ involvement (cardiac, renal, respiratory, hematological, gastrointestinal, dermatological or neurological) AND
- no alternative plausible diagnoses are available AND
- positive for a current or recent SARS-CoV-2 infection (detection by RT-PCR, serology or Antigen test or COVID-19 exposure within 4 weeks prior to the onset of symptoms).

Therapeutic Administration and Formulations

The invention provides therapeutic compositions comprising the antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like.

A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA.

These formulations include, for example, aqueous solutions, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax.

The dose may vary depending upon the age and the size of a subject to be administered, severity of the target disease, individual condition of the subject, route of administration, and the like.

The antibody and antigen-binding fragment thereof is used for treating various conditions and diseases associated with SARS-CoV-2, including fever, cough, fatigue, shortness of breath, loss

of smell and taste, pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD), diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis, plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC), leukoerythroblastic reaction, microvesicular steatosis in the liver and any combination thereof.

Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted.

Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis.

Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, inhalative and oral routes.

The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome.

In certain embodiments, the pharmaceutical composition can be delivered in a controlled release system, such as a pre-filled syringe, a dermal patch, infusion, pen- or auto-injector-device (single-dose, multi-dose, disposable, re-usable), syrette, large-volume pump-device, and the like.

In one embodiment a pre-filled syringe, or auto-injector, or pump device is preferred.

In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc.

These injectable preparations may be prepared by methods publicly known.

For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections.

As the aqueous solution for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc.

As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe.

In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused.

In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device.

Once the reservoir is emptied of the composition, the entire device is discarded.

Numerous reusable pen and auto-injection delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DI-SETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few.

Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly).

Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients.

Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

The invention provides therapeutic methods in which the antibody or antibody fragment of the invention is useful to treat COVID-19 associated with a variety of conditions involving SARS-CoV-2. The antibodies or antigen-binding fragments of the invention are particularly useful for the treatment of the viral infection with SARS-CoV-2.

Combination therapies may include the antibodies or antigen-binding fragments of the invention with, for example, one or more of any agent that

- (1) prevents or reduces the infectivity of SARS-CoV-2;
- (2) prevents or reduces secondary infections with other viruses (especially respiratory viruses such as influenza), bacteria (especially bacteria residing in the respiratory tract of the host) and/or parasites;
- (3) prevents or reduces any overreaction of the immune-system of the host (e.g. ADE-reaction or cytokine storm syndrome)
- (4) prevents or reduces any conditions associated with COVID-19, such as fever, cough, fatigue, shortness of breath, loss of smell and taste, pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD), diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC),

leukoerythroblastic reaction, microvesicular steatosis in the liver and any combination thereof.

Examples of such therapeutic agents to be used in combination with the antibodies or antigen-binding fragments of the invention in a pharmaceutical composition include, but are not limited to antiviral drugs including Indinavir, Saquinavir, Lopinavir, Ritonavir, interferone-beta, Remdesivir, Favipiravir, Oseltamivir, Chloroquin, Hydroxychloroquin, Umifenovir; serine proteases, including TMPRSS2 or Camostatmesilat; antibiotics, including Linezolid, Azithromycin, Nemonoxacin or Fluorchinolone; interleukin-6-rezeptor-antagonists, including Tocilizumab or Sarilumab in mono-therapy or combined with Methotrexate; anti-parasite treatments, including Ivermectin; anticoagulants and any combination thereof.

In a further embodiment the antibodies or antigen-binding fragments of the invention in a pharmaceutical composition may be combined with at least one member of the group comprising Tocilizumab, Dexamethasone, Remdesivir, Lopinavir, Hydroxychloroquine, Hydrocortisone, Methylprednisolone, Prednisolone, antibiotics, and any combination thereof.

The term “melting temperature of a protein (T_m)” as used herein means a value which is a thermodynamic parameter of a protein that can only strictly be determined under thermodynamic equilibrium conditions and with well-known baseline parameters for the required thermodynamic fit. However, thermal unfolding experiments are often not in equilibrium, because irreversible protein aggregation processes keep removing unfolded protein from the reaction, making the unfolding process irreversible. Heating rates faster than 1°C/min additionally decrease the equilibrium-resemblance of the unfolding reaction. T_{m1} as used herein refers to the unfolding of the a first domain (for example Fc) of the tested antibody; T_{m2} as used herein refers to the unfolding of a second domain (for example Fab) of the tested antibody.

The term “onset temperature of thermal melting ($T_{m\text{ onset}}$)” as used herein means the temperature at which thermal protein unfolding starts. It is determined from the first derivative curve of the thermal unfolding signal (either 350/330 nm ratio signal or individual 350 and 330 nm signals).

The term “Inflection point (IP)” as used herein means that due to constraints, in nanoDSF, thermal melting temperatures of proteins are determined directly from the measured data by using a smoothing algorithm and detection of inflection points in the data. Assuming an equilibrium, determined IPs correspond to the T_m values of protein.

The term “onset temperature of protein aggregation (T_{agg})” as used herein means the temperature at which the protein sample starts to aggregate. It is determined from the first derivate curve of the back-scattering signal. Typically, T_{agg} correlates with T_m . For proteins that show

more than one unfolding event, aggregation may start as a consequence of the first unfolding event or of later ones.

The term “ratio 350/330” as used herein means the measure for a spectral shift in the fluorescence emission profile of the Tryptophan (Trp) residues. This shift occurs in most unfolding events and is caused by the environmental change that the Trp residues undergo upon unfolding of the protein: In the folded state, Trp residues are often buried in the hydrophobic core of a protein, which leads to the fluorescence emission peaking around 330 nm. They then become surface-exposed during unfolding, which often shifts the fluorescence emission peak toward 350 nm. Since the ratio 350 nm / 330 nm typically obliterates effects of auto-fluorescent additives and general fluorescence decay with increasing temperature, this detection mode is usually more robust than single wavelength detection. Therefore, clear IP points and T_m s can be derived which might not be visible in the single wavelength detection modes. Vice versa, it is also possible that unfolding events which do not trigger an emission peak shift are visible in the single wavelength data, but not in the ratio.

The term “scattering” as used herein means the phenomenon that any particle struck by light will reradiate its energy in all directions with an intensity depending on the direction. The intensity of light passing through a sample containing particles (e.g. aggregates) will decrease as the light encounters each particle and gets scattered. The Prometheus™ system measures this using backreflection optics in order to understand a sample’s aggregation behavior.

The term “germinality index” (GI) describes herein the percentage of identity to the IMGT human germline gene. CDR3 is excluded from the analysis, since it is not encoded in the germline gene. For VH a homology of >90.8% the tool score is +1 and for a homology <80.7% the tool score is -1. For VL a homology of >95.1% the tool score is +1 and for a homology <85.5% the tool score is -1.

The term “hydrophobicity score” as used herein is calculated and summed to total score (similar to SAP (Spatial-Aggregation-Propensity) algorithm; Chennamsetty, 2009. For the total hydrophobicity score the scores of all VH and VL residues are summed up if they are >0.05 (hydrophobic and on the surface). The tool score is +1 for a total score < 5.1 and the tool score is -1 for a total score > 5.1. For the total hydrophobicity score the scores of all CDR residues of VH and VL (Kabat annotation) are summed up if they are >0.05 (hydrophobic and on the surface). The tool score is +1 for a CDR score < 2.3 and the tool score is -1 for a CDR score >2.5.

The term pI (isoelectric point) as used herein is the pH at which the net charge of a protein is zero. For polypeptides, the isoelectric point depends primarily on the dissociation constants

(pKa) for the ionizable groups of seven charged amino acids: Glutamate (δ -carboxyl group), aspartate (β -carboxyl group), cysteine (thiol group), tyrosine (phenol group), histidine (imidazole side chains), lysine (ϵ -ammonium group), arginine (guanidinium group) and terminal groups (NH₂ and COOH). The tool uses the EMBOSS (Rice, 2000) pKa values:

NH ₂	COOH	C	D	E	H	K	R	Y
8,6	3,6	8,5	3,9	4,1	6,5	10,8	12,5	10,1

A protein has its lowest solubility at its pI and for antibodies it is generally observed that increases in net positive charge of antibodies result in increased blood clearance and increased tissue retention with shorter half-life, whereas with lower pI antibodies have decreased tissue uptake and longer half-life. Therefore antibodies with a pI < 7.6 or > 8.4 get a tool score of -1 and antibodies with a pI between 7.6 - 8.4 get a tool score of +1.

The term “surface charge” as used herein is the prediction of the surface charge the hydrophobicity values of all amino acids changed to charge values:

D and E have a value of -1, K and R have a value of 1, H has a value of 0.1 and all other amino acids have a value of 0. For all surface residues the charge score is calculated.

For the scFv charge score the scores of all surface D, E, K, R, H residues of VH and VL are summed up. The tool score is +1 for a total charge score between -4.5 and 3.5 and the tool score is -1 for a total score < -4.5 and >3.5.

For the total CDR charge score the scores of all D, E, K, R, H residues of VH and VL CDRs are summed up. The tool score is +1 for a total CDR charge score between -2.1 and 1 and the tool score is -1 for a total score < -2.1 and >1.

For the negative CDR charge score the scores of all D, E residues of VH and VL CDRs are summed up. The tool score is +1 for a negative CDR charge score > -3.5 and the tool score is -1 for a total score < -4.5.

For the positive CDR charge score the scores of all D, E residues of VH and VL CDRs are summed up. The tool score is +1 for a positive CDR charge score < 2.5 and the tool score is -1 for a total score < 3.

In one aspect of the present invention the antibody or antigen-binding fragment or a substantially similar variant thereof can be used for a test-assay, either in a laboratory setup (e.g. a serological test for SARS-CoV-2 antigen) and/or in a quick-test-format.

In one aspect the present invention pertains to a humanized or a human therapeutic monoclonal

antibody or antigen-binding fragment, or a substantially similar variant thereof, that binds specifically to SARS-CoV-2 glycoprotein S with the amino acid sequence of SEQ ID NO: 02 and neutralizes the infectious activity of SARS-CoV-2.

In another aspect the antibody or antigen-binding fragment or a substantially similar variant thereof is monoclonal.

In yet another aspect the antibody or antigen-binding fragment or a substantially similar variant thereof is a humanized or a human monoclonal antibody, or humanized or a human antigen-binding fragment thereof.

In another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof shows a neutralization of the infectious activity of SARS-CoV-2 which results in confluence score of at least 95% as measured with an infectious SARS-CoV-2 VERO E6 neutralizing assay.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof has a specific binding to the RBD with a K_D of 65×10^{-9} M (65 nM) or less

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to an epitope within the receptor binding domain (RBD) of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO: 06) and binds specifically to at least one of the escape-mutations of the S1 domain of SARS-CoV-2 glycoprotein selected from the list consisting of S1-V367F, S1-N439K, S1-G476S, S1-V483A, S1-E484K, S1-G485R, S1-F486V, S1-7PM-mutant (SEQ ID NO: 1730), and any combination thereof.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically both to an epitope comprising the amino acids 400-FVIRGDEVQRQIAPQTGKIADDYN-422 (SEQ ID NO: 1820) within the RBD (SEQ ID NO: 06) as well as to the S1-7PM-mutant (SEQ ID NO: 1730).

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to an epitope within the RBD (SEQ ID NO: 06) with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928; and wherein the contamination ratio is less than 0.2.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof has a contamination ratio of 0.1 or less, of 0.05 or less, of 0.025 or less or even of 0.001 or less.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 165-AKRVNCYFPLQSYGFQ-180 (SEQ ID NO: 1821) within the S1-7PM-mutant (SEQ ID NO: 1730) with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305; and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309 or with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect the antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the RBD-domain of the S1 domain of SARS-CoV-2 glycoprotein of SEQ ID No: 06 with the ACE2-receptor of the sequence depicted in SEQ ID NO: 08 in a competition assay.

In yet another aspect the antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to an epitope within an amino acid sequence selected from the group of SEQ ID No: 04 and/or SEQ ID No: 06 with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406; and a light chain variable region of the amino acid sequence in SEQ ID NO: 408.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof comprises:

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 910, 350, 390, and 1306;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 912, 352, 392, and 1307;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 914, 354, 394, and 1308;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 916, 356, 396, and 1310;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 918, 358, 398, and 1311;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 920, 360, 400, and 1312.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof is selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof comprises a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof comprises:

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 210, 230, 250, 270, 290, 310, 330, 350, 370, 390, 410, 430, 450, 470, 490, 510, 530, 550, 570, 590, 610, 630, 650, 670, 690, 710, 730, 750, 770, 790, 810, 830, 850, 870, 890, 910, 930, 950, 970, 990, 1110, 1130, 1150, 1170, 1190, 1210, 1230, 1250, 1270, 1290, 1298, 1306, 1314, 1322, 1330, 1338, 1346, 1354, 1362, 1370, 1378, 1386, 1394, 1402, 1410, 1418, 1426, 1434, 1442, 1450, 1458, 1466, 1474, 1482, 1490, 1498, 1506, 1514, 1522, 1530, 1538, 1546, 1554, 1562, 1570, 1578, 1586, 1594, 1602, 1610, 1618, 1626, 1634, 1642, 1650, 1658, 1666, 1674, 1682, 1690, 1698, 1706, 1714, and 1722;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 32, 52, 72, 92, 112, 132, 152, 172, 192, 212, 232, 252, 272, 292, 312, 332, 352, 372, 392, 412, 432, 452, 472, 492, 512, 532, 552, 572, 592, 612, 632, 652, 672, 692, 712, 732, 752, 772, 792, 812, 832, 852, 872, 892, 912, 932, 952, 972, 992, 1112, 1132, 1152, 1172, 1192, 1212, 1232, 1252, 1272, 1291, 1299, 1307, 1315, 1323, 1331, 1339, 1347, 1355, 1363, 1371, 1379, 1387, 1395, 1403, 1411, 1419, 1427, 1435, 1443, 1451, 1459, 1467, 1475, 1483, 1491, 1499, 1507, 1515, 1523, 1531, 1539, 1547, 1555, 1563, 1571, 1579, 1587, 1595, 1603, 1611, 1619, 1627, 1635, 1643, 1651, 1659, 1667, 1675, 1683, 1691, 1699, 1707, 1715, and 1723;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence selected from the

group consisting of SEQ ID NO: 14, 34, 54, 74, 94, 114, 134, 154, 174, 194, 214, 234, 254, 274, 294, 314, 334, 354, 374, 394, 414, 434, 454, 474, 494, 514, 534, 554, 574, 594, 614, 634, 654, 674, 694, 714, 734, 754, 774, 794, 814, 834, 854, 874, 894, 914, 934, 954, 974, 994, 1114, 1134, 1154, 1174, 1194, 1214, 1234, 1254, 1274, 1292, 1300, 1308, 1316, 1324, 1332, 1340, 1348, 1356, 1364, 1372, 1380, 1388, 1396, 1404, 1412, 1420, 1428, 1436, 1444, 1452, 1460, 1468, 1476, 1484, 1492, 1500, 1508, 1516, 1524, 1532, 1540, 1548, 1556, 1564, 1572, 1580, 1588, 1596, 1604, 1612, 1620, 1628, 1636, 1644, 1652, 1660, 1668, 1676, 1684, 1692, 1700, 1708, 1716, and 1724;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 36, 56, 76, 96, 116, 136, 156, 176, 196, 216, 236, 256, 276, 296, 316, 336, 356, 376, 396, 416, 436, 456, 476, 496, 516, 536, 556, 576, 596, 616, 636, 656, 676, 696, 716, 736, 756, 776, 796, 816, 836, 856, 876, 896, 916, 936, 956, 976, 996, 1116, 1136, 1156, 1176, 1196, 1216, 1236, 1256, 1276, 1294, 1302, 1310, 1318, 1326, 1334, 1342, 1350, 1358, 1366, 1374, 1382, 1390, 1398, 1406, 1414, 1422, 1430, 1438, 1446, 1454, 1462, 1470, 1478, 1486, 1494, 1502, 1510, 1518, 1526, 1534, 1542, 1550, 1558, 1566, 1574, 1582, 1590, 1598, 1606, 1614, 1622, 1630, 1638, 1646, 1654, 1662, 1670, 1678, 1686, 1694, 1702, 1710, 1718, and 1726;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 38, 58, 78, 98, 118, 138, 158, 178, 198, 218, 238, 258, 278, 298, 318, 338, 358, 378, 398, 418, 438, 458, 478, 498, 518, 538, 558, 578, 598, 618, 638, 658, 678, 698, 718, 738, 758, 778, 798, 818, 838, 858, 878, 898, 918, 938, 958, 978, 998, 1118, 1138, 1158, 1178, 1198, 1218, 1238, 1258, 1278, 1295, 1303, 1311, 1319, 1327, 1335, 1343, 1351, 1359, 1367, 1375, 1383, 1391, 1399, 1407, 1415, 1423, 1431, 1439, 1447, 1455, 1463, 1471, 1479, 1487, 1495, 1503, 1511, 1519, 1527, 1535, 1543, 1551, 1559, 1567, 1575, 1583, 1591, 1599, 1607, 1615, 1623, 1631, 1639, 1647, 1655, 1663, 1671, 1679, 1687, 1695, 1703, 1711, 1719, and 1727;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840, 860, 880, 900, 920, 940, 960, 980, 1000, 1120, 1140, 1160, 1180, 1200, 1220, 1240, 1260, 1280, 1296, 1304, 1312, 1320, 1328, 1336, 1344, 1352, 1360, 1368, 1376, 1384, 1392, 1400, 1408, 1416, 1424, 1432, 1440, 1448, 1456, 1464, 1472, 1480, 1488, 1496, 1504, 1512, 1520, 1528, 1536, 1544, 1552, 1560, 1568, 1576, 1584, 1592, 1600, 1608, 1616, 1624, 1632, 1640, 1648,

1656, 1664, 1672, 1680, 1688, 1696, 1704, 1712, 1720, and 1728.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof comprises a variable heavy chain (V_H) with an amino acid sequence selected from the group consisting of SEQ ID NO: 24, 44, 64, 84, 104, 124, 144, 164, 184, 204, 224, 244, 264, 284, 304, 324, 344, 364, 384, 404, 424, 444, 464, 484, 504, 524, 544, 564, 584, 604, 624, 644, 664, 684, 704, 724, 744, 764, 784, 804, 824, 844, 864, 884, 904, 924, 944, 964, 984, 1004, 1024, 1044, 1064, 1084, 1104, 1124, 1144, 1164, 1184, 1204, 1224, 1244, 1264, 1284, 1289, 1297, 1305, 1313, 1321, 1329, 1337, 1345, 1353, 1361, 1369, 1377, 1385, 1393, 1401, 1409, 1417, 1425, 1433, 1441, 1449, 1457, 1465, 1473, 1481, 1489, 1497, 1505, 1513, 1521, 1529, 1537, 1545, 1553, 1561, 1569, 1577, 1585, 1593, 1601, 1609, 1617, 1625, 1633, 1641, 1649, 1657, 1665, 1673, 1681, 1689, 1697, 1705, 1713, and 1721;

and

a variable light chain (V_L) selected from the group consisting of SEQ ID NO: 22, 42, 62, 82, 102, 122, 142, 162, 182, 202, 222, 242, 262, 282, 302, 322, 342, 362, 382, 402, 422, 442, 462, 482, 502, 522, 542, 562, 582, 602, 622, 642, 662, 682, 702, 722, 742, 762, 782, 802, 822, 842, 862, 882, 902, 922, 942, 962, 982, 1002, 1022, 1042, 1062, 1082, 1102, 1122, 1142, 1162, 1182, 1202, 1222, 1242, 1262, 1282, 1293, 1301, 1309, 1317, 1325, 1333, 1341, 1349, 1357, 1365, 1373, 1381, 1389, 1397, 1405, 1413, 1421, 1429, 1437, 1445, 1453, 1461, 1469, 1477, 1485, 1493, 1501, 1509, 1517, 1525, 1533, 1541, 1549, 1557, 1565, 1573, 1581, 1589, 1597, 1605, 1613, 1621, 1629, 1637, 1645, 1653, 1661, 1669, 1677, 1685, 1693, 1701, 1709, 1717, and 1725.

The therapeutic antibody or antigen-binding fragment thereof comprises a heavy chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 26, 46, 66, 86, 106, 126, 146, 166, 186, 206, 226, 246, 266, 286, 306, 326, 346, 366, 386, 406, 426, 446, 466, 486, 506, 526, 546, 566, 586, 606, 626, 646, 666, 686, 706, 726, 746, 766, 786, 806, 826, 846, 866, 886, 906, 926, 946, 966, 986, 1006, 1026, 1046, 1066, 1086, 1106, 1126, 1146, 1166, 1186, 1206, 1226, 1246, 1266, and 1286;

and

a light chain with an amino acid sequence selected from the group consisting of SEQ ID NO: 28, 48, 68, 88, 108, 128, 148, 168, 188, 208, 228, 248, 268, 288, 308, 328, 348, 368, 388, 408, 428, 448, 468, 488, 508, 528, 548, 568, 588, 608, 628, 648, 668, 688, 708, 728, 748, 768, 788, 808, 828, 848, 868, 888, 908, 928, 948, 968, 988, 1008, 1028, 1048, 1068, 1088, 1108, 1128, 1148, 1168, 1188, 1208, 1228, 1248, 1268, and 1288.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof is characterized by one or more of the following:

- I. capable of binding specifically to the S1- domain of SARS-CoV-2 Glycoprotein with an affinity (K_D) of 1.5×10^{-8} M or less, preferably less than 3×10^{-9} M,
- II. capable of reducing or preventing an antibody-dependent enhancement (ADE) reaction in a patient,
- III. capable of preventing, reducing or healing a viral infection with SARS-CoV-2,
- IV. capable of reducing or preventing the cytokine storm syndrome (CSS) in a patient,
- V. capable of reducing the load of viral particles in the sputum of a patient by at least 50% measured 48 hours after administration of the therapeutic antibody or antigen-binding fragment thereof.
- VI. having an IC_{50} of less than 0.036 $\mu\text{g/ml}$, preferably of 0.026 $\mu\text{g/ml}$ or less.

In yet another aspect an isolated nucleic acid molecule encoding the therapeutic antibody or antigen-binding fragment is encompassed.

In yet another aspect an expression vector is encompassed comprising said nucleic acid molecule.

In yet another aspect a method of producing the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof is encompassed comprising the steps of

- i. introducing the expression vector mentioned before into an isolated host cell,
- ii. growing the cell under conditions permitting production of the antibody or antigen-binding fragment thereof ,
- iii. recovering the antibody or fragment so produced.

In yet another aspect a pharmaceutical composition is encompassed comprising said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof.

In yet another aspect said pharmaceutical composition further comprises at least a second therapeutic agent, wherein the second therapeutic agent is selected from antiviral drugs including Indinavir, Saquinavir, Lopinavir, Ritonavir, interferon-beta, Remdesivir, Favipiravir, Oseltamivir,

Chloroquin, Hydroxychloroquin, Umifenovir; serine proteases, including TMPRSS2 or Camostatmesilat; antibiotics, including Linezolid, Azithromycin, Nemonoxacin or Fluorchinolone; interleukin-6-receptor-antagonists, including Tocilizumab or Sarilumab in mono-therapy or combined with Methotrexate; anti-parasite treatments, including Ivermectin; anticoagulants and any combination thereof.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to present invention is for the use in the treatment of a SARS-CoV-2-related disease or condition in a patient.

In yet another aspect said SARS-CoV-2-related disease or condition is selected from the group comprising fever, cough, fatigue, shortness of breath, loss of smell and taste, pleurisy, pericarditis, lung consolidation, pulmonary oedema, pneumonia, serous exudation, fibrin exudation, pulmonary oedema, pneumocyte hyperplasia, large atypical pneumocytes, interstitial inflammation with lymphocytic infiltration and multinucleated giant cell formation, diffuse alveolar damage (DAD), diffuse alveolar exudates, acute respiratory distress syndrome (ARDS), severe hypoxemia, exudates in alveolar cavities and pulmonary interstitial fibrosis plasmocytosis in bronchoalveolar lavage (BAL), disseminated intravascular coagulation (DIC), leukoerythroblastic reaction, microvesicular steatosis in the liver, any symptom associated with COVID-19 and any combination thereof.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to the present invention competes for binding to an epitope within an amino acid sequence selected from the group of SEQ ID No: 04 and/or SEQ ID No: 06 with an antibody selected from a group comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 906 and a light chain variable region of the amino acid sequence in SEQ ID NO: 908; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1066 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1068; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1166 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1168; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In one embodiment said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to at least one epitope within the S1-7PM-mutant (SEQ ID NO.: 1730), selected from 365-YSFLY-369 (SEQ ID NO: 1822), 436-WNSKNLD-443 (SEQ ID NO: 1823), and/or 474-QASSTPCNGAKRVNCY-489 (SEQ ID NO: 1824) (the numbering of the residues corresponds to the numbering of the amino acids within the S1 domain of SARS-CoV-2 glycoprotein S, i.e. SEQ ID NO: 02).

In yet another embodiment said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least one epitope within the S1-7PM-mutant, selected from 365-YSFLY-369 (SEQ ID NO: 1822), 436-WNSKNLD-443 (SEQ ID NO: 1823), and/or 474-QASSTPCNGAKRVNCY-489 (SEQ ID NO: 1824) (the numbering of the residues corresponds to the numbering of the amino acids within the S1 domain of SARS-CoV-2 glycoprotein S, i.e. SEQ ID NO: 02) with the ACE2-rezeptor.

In yet another embodiment therapeutic said antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to and/or competes for binding to at least two and/or all three epitopes within the S1-7PM-mutant, selected from 365-YSFLY-369 (SEQ ID NO: 1822), 436-WNSKNLD-443 (SEQ ID NO: 1823), and/or 474-QASSTPCNGAKRVNCY-489 (SEQ ID NO: 1824) (the numbering of the residues corresponds to the numbering of the amino acids within the S1 domain of SARS-CoV-2 glycoprotein S, i.e. SEQ ID NO: 02) with the ACE2-rezeptor.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 483-VEGFNCYF-490 (SEQ ID NO: 1825) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein S with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 483-VEGFNCYF-490 (SEQ ID NO: 1825) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein S with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially

similar variant thereof competes for binding to the epitope 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 400-FVIRGDEVQRQIAPQTGKIADYN-422 (SEQ ID NO: 1826) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 400-FVIRGDEVQRQIAPQTGKIADYN-422 (SEQ ID NO: 1826) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least one or two or three or more of the epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQIAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein S with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least one or two or three or more of the epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQIAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein S with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the S1-7PM-mutant (SEQ ID NO: 1730) of the S1 domain of SARS-CoV-2 glycoprotein with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the S1-7PM-mutant (SEQ ID NO: 1730) of the S1 domain of SARS-CoV-2 glycoprotein with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another embodiment said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least two and/or all three epitopes within the S1-7PM-mutant, selected from 365-YSFLY-369 (SEQ ID NO: 1822), 436-WNSKNLD-443 (SEQ ID NO: 1823), and/or 474-QASSTPCNGAKRVNCY-489 (SEQ ID NO: 1824) (the numbering of the residues corresponds to the numbering of the amino acids within the S1 domain of SARS-CoV-2 glycoprotein S, i.e. SEQ ID NO: 02) with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another embodiment said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least two and/or all three epitopes within the S1-7PM-mutant, selected from 365-YSFLY-369 (SEQ ID NO: 1822), 436-WNSKNLD-443 (SEQ ID NO: 1823), and/or 474-QASSTPCNGAKRVNCY-489 (SEQ ID NO: 1824) (the numbering of the residues corresponds to the numbering of the amino acids within the S1 domain of SARS-CoV-2 glycoprotein S, i.e. SEQ ID NO: 02) with an antibody selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to at least one, or two, or three, or more of the epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGGNYYLYRLFRK-SNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQIAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein, and binds specifically to at least one, or two, or three, or more of the

epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827).

In yet another aspect said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to at least one, or two, or three, or more of the epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein, and competes for binding to at least one, or two, or three, or more of the epitopes selected from 483-VEGFNCYF-490 (SEQ ID NO: 1825), 437-NSNNLDSKVGGNYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811), 400-FVIRGDEVQRQAPQTGKIADYN-422 (SEQ ID NO: 1826), and/or 470-TEIYQAGSTPC-480 (SEQ ID NO: 1827) with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926; and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to the present invention comprises at least one of the CDRs selected from

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence of SEQ ID NO: 1306;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence of SEQ ID NO: 1307;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence of SEQ ID NO: 1308;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence of SEQ ID NO: 1310;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence of SEQ ID NO: 1311;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence of SEQ ID NO: 1312.

In yet another aspect of the invention said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to an epitope within the receptor binding domain (RBD) of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO: 06) and binding specifically to the S1-7PM-mutant of SEQ ID No: 1730, with an antibody comprising a heavy

chain variable region of the amino acid sequence in SEQ ID NO: 1305; and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309 or with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect of the invention said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof competes for binding to the epitope 483-VEGFNCYFPLQSYGFQPTNGV-505 (SEQ ID NO: 1828) within the RBD (SEQ ID NO.: 6) and to the S1-7PM-protein of SEQ ID No.: 1730 with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305; and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309 or with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the epitope 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811) within the RBD (SEQ ID NO.: 6) and competes for binding to 483-VEGFNCYF-490 (SEQ ID NO: 1825) within the RBD of the S1 domain of SARS-CoV-2 glycoprotein and binds specifically to the S1-7PM-mutant (SEQ ID NO: 1730) of the S1 domain of SARS-CoV-2 glycoprotein.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the epitope 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811) within the RBD (SEQ ID NO.: 6) with a K_D of 70 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and shows a contamination ratio of 0.2 or less.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the RBD of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO.: 6) with a K_D of 70 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and shows a contamination ratio of 0.2 or less.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the RBD of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO.: 6) with a K_D of 20 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and shows a contamination ratio of 0.1 or less.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a

substantially similar variant thereof binds specifically to the RBD of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO.: 6) with a K_D of 70 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and binds specifically to the S1-7PM-mutant (SEQ ID NO: 1730) with a K_D of 70 nM or less and shows a contamination ratio of 0.2 or less.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the RBD of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO.: 6) with a K_D of 10 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and binds specifically to the S1-7PM-mutant (SEQ ID NO: 1730) with a K_D of 45 nM or less (antibody against S1-7PM was immobilized on a Protein A coated biosensor) and shows a contamination ratio of 0.1 or less.

In yet another aspect of the invention the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof binds specifically to the epitope 437-NSNNLDSKVGG-NYNYLYRLFRKSNLKP-463 (SEQ ID NO: 1811) within the RBD (SEQ ID NO.: 6) with a K_D of 70 nM or less ("wild-type" RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface) and binds specifically to the S1-7PM-mutant (SEQ ID NO: 1730) with a K_D of 45 nM or less (antibody against S1-7PM was immobilized on a Protein A coated biosensor) and shows a contamination ratio of 0.1 or less.

In yet another aspect therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof is characterized by one or more of the following:

- capable of binding specifically to the RBD of SARS-CoV-2 Glycoprotein S with an affinity (KD) of 6.5×10^{-8} M or less, preferably less than 10×10^{-9} M,
- capable of reducing or preventing an antibody-dependent enhancement (ADE) reaction in a patient,
- capable of preventing, reducing or healing a viral infection with SARS-CoV-2,
- capable of reducing or preventing the cytokine storm syndrome (CSS) in a patient,
- capable of reducing the load of viral particles in the sputum of a patient by at least 50% for at least 48 hours after administration of the therapeutic antibody or antigen-binding fragment thereof,
- having a specific binding specifically to the S1-7PM-mutant (SEQ ID. 1730) with a

KD of less than 3×10^{-9} M,

- having an IC₅₀ of less than 0.036 µg/ml, preferably of 0.026 µg/ml or less.

In another aspect the present invention pertains to an isolated nucleic acid molecule encoding said therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof.

In another aspect the present invention pertains to an expression vector comprising said nucleic acid molecule.

In another aspect the present invention pertains to a method of producing the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof comprising the steps of

introducing said expression vector according into an isolated host cell,

growing the cell under conditions permitting production of the antibody or antigen-binding fragment thereof ,

recovering the antibody or fragment so produced.

In one embodiment the antibody may be produced in the animal and/or human body, e.g. in a body cell, by using for example a stabilized mRNA, DNA, plasmid, viral vector or other vector enabling the production of the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof within the animal or human body.

In another aspect the present invention pertains to a pharmaceutical composition comprising the therapeutic antibody or antigen-binding fragment or a substantially similar variant, and a pharmaceutically acceptable carrier.

In another aspect the present invention pertains to a therapeutic antibody or antigen-binding fragment for the use in the prevention and/or treatment of a SARS-CoV-2-related disease or condition in a patient.

Short Description of the Figures

Figure 1a – Representative depiction of a SEC-HPLC-measurement of a therapeutic antibody according to the present invention. A single peak indicates a high purity (i.e. low contamination) of the antibody. In the depiction is only the peak of the monomer visible.

Figure 1b - Representative depiction of a SEC-HPLC-measurement of a regular antibody in the prior art. Several peaks indicate the contamination with multimers and dimers besides the monomer. Such an antibody would not be suitable to be used as a therapeutic antibody due to undesired stimulatory effects on the host's immune system.

Figure 2 – Result of an infectious SARS-CoV-2 neutralizing assay (Confluent VERO E6-6-Test) with different antibodies. The higher the confluence the better the neutralizing activity of the antibody. For therapeutic purposes a confluence of at least 95% is desired.

Figure 3 – List of some of the antibodies of the present invention with germline VH and VL as well as their CDR-sequences. Depicted are *inter alia* the therapeutic antibodies: YU505-A01, YU505-A02, YU505-E01, YU505-F05, YU534-C12, YU534-D09, YU535-A02, YU536-D04, YU537-H08 and YU537-H11. YU505-A02, YU536-D04 and YU537-H11 are preferred therapeutic antibodies.

Figure 4 – Representation of the amino acid sequence of the VL and VH of the therapeutic antibodies YU505-A01 (a), YU505-A02 (b), YU505-E01 (c) and YU505-F05 (d). YU505-A02 (b) is useful both as a therapeutic antibody as well as a reference antibody. Any antibody competing with YU505-A02 (b) to the target epitope(s) is to be considered a useful therapeutic antibody according to the present invention.

Figure 5 – Representation of the scFv-sequences of antibody-fragments from YU505-A01 (a), YU505-A02 (b), YU505-E01 (c) and YU505-F05 (d).

Figure 6 – Representation of a test for “stickiness” of several therapeutic antibodies as compared with known antibodies. The lower the signal the smaller is the unspecific binding of the antibodies to undesired targets.

Figure 7 – Representation of a SEC-HPLC-measurement of the therapeutic antibodies YU505-A01 (a), YU505-A02 (b), YU505-E01 (c) and YU505-F05 (d). All antibodies show a high purity with a contamination ratio of well below 1. The peaks at time-points > 10 are due to impurities of the column and not associated with the antibodies.

Figure 8 – Exemplary results of ACE2:RBD competition assay of some antibodies of the present

invention at different concentrations. 100% represents no competition, 0% represents full competition. A competition of less than 70% at 0.5 µg/ml was considered necessary to qualify an antibody with sufficient activity for further testing.

Figure 9 – Depiction of the contamination ratio (i.e. multimerization) of a selection of antibodies of the present invention. Measurement was done by SEC-HPLC and the multimerization was compared with IVIGs. A contamination ratio of less than 1 was considered necessary to qualify an antibody for further testing.

Figure 10 – Depiction of the specificity of a selection of therapeutic human antibodies according to the invention. Note: The binding specifically to the different negative antigens was normalized to the binding of avelumab (a fully human monoclonal antibody medication for the treatment of Merkel cell carcinoma, urothelial carcinoma, and renal cell carcinoma).

Figure 11 – Depiction of effective neutralization of the virus and blocking of the entry of virus into mammalian cells at 1 µg/ml with different antibodies of the present invention. All depicted antibodies show sufficient neutralization to be considered for therapeutic use.

Figure 12 – Depiction of the effective blocking of the ACE2:RBD interaction site for a selection of inventive antibodies (all inventive antibodies were tested, data not shown). A blocking of at least 50% of the ACE2:RBD interaction site was considered necessary to qualify an antibody for further testing.

Figure 13 – Depiction of the effective IC₅₀-binding specifically to the RBD in subnanomolar range for a selection of inventive antibodies (all inventive antibodies were tested, data not shown). An IC₅₀ of less than 0,2 nM was considered necessary to qualify an antibody for further testing. For YU505-A01 no saturation ELISA curve was detected.

Figure 14 – Depiction of the heat stability test determined by NanoDSF for a selection of inventive antibodies (all inventive antibodies were tested, data not shown). Melting T ranges from about 60°C to about 70°C. Aggregation T ranges from about 68°C to 81°C. An melting T of at least 65°C and aggregation T of at least 70°C was considered necessary to qualify an antibody for further testing.

Figure 15 – Depiction of the multimer-formation under stress conditions for a selection of inventive antibodies (all inventive antibodies were tested, data not shown). No increased multimer-formation of above 4% could be detected showing the overall suitability in terms of purity and stability of the inventive antibodies..

Figure 16 – IC₅₀ measurement in a VERO E6 cell assay for a number of antibodies according to the present invention (all inventive antibodies were tested, data not shown). YU536-D04 and

YU537-H11 show a preferred IC₅₀-profile.

Figure 17 – Table depicting the results of the IC₅₀ measurement in a VERO E6 cell assay for a number of antibodies according to the present invention (all inventive antibodies were tested, data not shown). An IC₅₀ of less than 0.05 was considered necessary to qualify an antibody for further testing.

Figure 18 – Depiction of the antibody subfamily distribution in the universal HAL9/10 library (according to Kügler et al., 2015), the in vivo distribution of subfamilies (according to Tiller et al., 2013) and the distribution of antibodies against S1 selected from HAL9/10. (A) Abundance of VH. (B) Abundance of Vk. (C) Abundance of Vλ.

Figure 19 – Depiction of the flow cytometry analysis to determine the inhibition efficacy. A) screening analysis to determine the inhibition efficacy of scFv-Fc antibodies on ACE2 positive cells using 1500 nM antibody and 50 nM spike protein (30:1 ratio). The antibodies chosen for later analysis are marked in colors. The antibodies STE73-9-G3 and STE73-7H10 (marked with *) were identical. B) IC₅₀ determination by flow cytometry using 50 nM S1-S2 trimer and 4.7 – 1500 nM antibodies. C) IC₅₀ determination by flow cytometry using 10 nM RBD and 0.03-1000 nM antibodies.

Figure 20 – The binding of 17 inhibiting antibodies were tested on RBD (with murine Fc part), S1 (with murine Fc part) or S1-S2 (with His tag) in titration ELISA to determine the EC₅₀. As controls, a control antibody with a murine Fc part (an antibody which binds specifically to c-myc), human HEK293 cell lysate, BSA and lysozyme was used.

Figure 21 - Depiction of the flow cytometry analysis to determine the inhibition efficacy of antibody combinations on ACE2 positive cells and 50 nM S1-S2 and 1500 nM as a single antibody, respectively 750 nM of each antibody in a combination.

Figure 22 - Depiction of the flow cytometry analysis to determine the inhibition efficacy.

Figure 23 - Depiction of the SEC data of antigen production in insect High Five cells (A-C) and mammalian Expi293F cells (D-E). The table (F) indicates the most likely conformation of the protein due to the retention volume of the peaks with the corresponding area in percentage.

Figure 24 - A) Depiction of the flow cytometry inhibition analysis of spike S1-S2 trimer (50 nM in relation to monomer) binding specifically to living cells expressing ACE2 blocked by 1500 nM antibodies and B) Depiction of RBD-mFc (10 nM in relation to monomer) binding specifically to living cells expressing ACE2 blocked by 100 nM antibody. The antibodies STE72-1G5, STE72-4C10, STE72-8E1 and STE73-6C8 were used as example. The background control are trans-

fectected ACE2 cells and the reference are ACE2 positive cells incubated with labeled spike protein.

Figure 25 - Depiction of a flow cytometry inhibition analysis. Comparison of the inhibition of spike protein and RBD by flow cytometry on ACE2 positive Expi293F cells using 1000 nM scFv-Fc and 50 nM spike protein, respectively RBD (20:1 ratio). A) Inhibition of RBD binding in comparison of Expi293F transiently expressing ACE2 and Calu-3 cells. B) Inhibition of spike S1-S2 binding in comparison of Expi293F transiently expressing ACE2 and Calu-3. As negative control, an antibody which binds specifically to N-cadherin of *Danio rerio* was used.

Figure 26 - Overview on inhibiting antibodies. V-genes were determined by VBASE2 (vbase2.org) (Mollova et al., 2010). The EC_{50} were measured on 30 ng immobilized RBD-mFc, S1-mFc, S1-S2-His (trimer) by ELISA. The IC_{50} was measured by flow cytometry using 50 nM (in relation to monomer) S1-S2 trimer, respectively 10 nM RBD and ACE2 positive cells. The molar ration of antibody binding site: S1-S2 or RBD is given for 50% inhibition. N.D.: not determinable.

Figure 27 - Depiction of the result of an infectious SARS-CoV-2 neutralizing assay (Confluent VERO E6-6-Test) with different antibodies

Figure 28 – Table depicting the results of the IC_{50} measurement in a VERO E6 cell assay for a number of antibodies according to the present invention.

Figure 29 – IC_{50} measurement in a VERO E6 cell assay for a number of antibodies according to the present invention. STE90-C11 shows a preferred IC_{50} -profile.

Figure 30 – a) The result of the binding study according to example 11 with antibody STE90-C11 of the present invention to the 8 “escape”-mutants. The binding was also compared to other reported antibodies CB6, RGN10933, RGN10987, CR3022. It could be shown that the inventive antibody STE90-C11 shows significant better binding specifically to all 7 single-point “escape”-mutants as compared to reference antibodies, and diminished, but still detectable binding specifically to the seven point mutation “S1-7PM”. b) Same measurement but the depiction is in nM-concentration.

Figure 31 – The result of the binding study of the S1-protein with the seven single-point “escape” mutants as well as the seven point mutation “S1-7PM” to the ACE2-domain. This shows that all “escape”-mutants still bind to ACE2 and therefore are able to infect host-cells despite a significant altered RBD.

Figure 32 – The result of the binding study according to example 11 with a selection of antibodies of the present invention to the 8 “escape”-mutants. The binding was also compared to other

reported antibodies CB6, RGN10933, RGN10987, CR3022. It could be shown that the inventive antibodies show significant better binding specifically to all 7 single-point “escape”-mutants as compared to reference antibodies, and partially diminished, but still detectable binding specifically to the seven point mutation “S1-7PM”.

Figure 33 – Depiction of the results of the “binning study” according to example 11. “COM1”, “COM2” and “COM3” refer to different bins (“BIN 1”, “BIN 2”, “BIN 3”), i.e. antibodies belonging to the same COM or bin compete with each other. All preferred therapeutic antibodies belong to “COM1” and compete with reference antibody YU505-A02.

Figure 34 – Depiction of the results of K_D – measurements of a number of antibodies according to the present invention as well as two comparative antibodies against different point mutations as well as the S1-7PM-mutation. The measurements were done as described in example 13 (BLI), the antibody was immobilized on a Protein A coated biosensor.

Figure 35 – EC_{50} -analysis with STE90-C11 against a plurality of different single, double and triple RBD-mutants. Most mutants were still bound significantly, however, a reduction of binding could be seen for mutants K417N, K417T, N501Y, E484K/N501Y, B.1.1.7.

Figure 36 – Results of the selection pressure experiment as described further in example 18. With the Palivizumab control the wild-type virus is the prevailing virus form, whereas with the antibody STE90-C11 the K417T-mutant prevailed. This indicates that growth of wild-type virus and other virus mutants was significantly suppressed by STE90-C11.

Figure 37 – Depiction of a surface depiction on interaction sites on the RBD. STE90-C11 shows a large interaction surface on the RBD (light grey), much bigger even than the interaction surface of ACE2 (dark grey). This explains, why STE90-C11 binds to most RBD-escape mutants. Furthermore most ACE2-interaction-sites are covered by the STE90-C11-interaction surface, thus explaining the good inhibition of ACE2-RBD-interaction and, ultimately, the good virus suppression.

Figure 38 – Binding study of YU536-D04 and YU537-H11 to some “critical mutants” which were not bound that good by STE90-C11. It turns out that YU536-D04 and YU537-H11 bind to N501Y and K417N-mutant with similar binding quality as to wild-type RBD.

Figures 39 – 42 – Results of animal studies in hamsters and mice as described in example 17.

Figure 39 – Viral load in lungs of hamsters was reduced from about 10^6 to about 10^4 after 3 days of infection and reduced from about 10^6 to about 10^2 after 5 days of infection when 37 mg/kg STE90-C11 was injected into the animals. Lower dosage of 3.7 mg/kg reduced also the viral load, but was not enough to eradicate the virus.

Figure 40 – Change of body weight of hamsters was used as health-indicator. Hamsters treated with 37 mg/kg STE90-C11 recovered quicker and earlier (already from day 3 after injection) and never lost as much weight as control groups. Since hamsters are prone to mild to moderate COVID-19 infections, this indicates that mild to moderate COVID-19 infections may be either avoided or at least attenuated and accelerated.

Figure 41 – Viral load in lungs of K18hACE2 mice was reduced from about 10^6 to about 10^2 after 5 days of infection both with 6 mg/kg as well as 60 mg/kg of STE90-C11 injected into the animals. Interestingly already the lower dosage of 6 mg/kg reduces the viral load sufficiently in mice. A) Control vs. 60 mg/kg STE90-C11; B) Control vs. 6 mg/kg STE90-C11.

Figure 42 – Change of body weight of K18hACE2 mice was used as health-indicator. Mice treated with 6, 30, 60, 120 mg/kg STE90-C11 recovered quicker and earlier (already from day 3 after injection) and never lost as much weight as control group (in which some mice died). Since K18hACE2 mice are prone to severe COVID-19 infections, this indicates that severe COVID-19 infections may be either avoided or at least attenuated and accelerated and chances of death are significantly reduced.

Figure 43 – Amino acid sequence alignment of heavy and light chain of the two antibodies STE90-C11 and YU536-D04 using Clustal™ software (version Omega). The high sequence identity shows that the antibodies of the invention form a structural and functional related cluster of antibodies with outstanding performance, especially with respect to the binding to escape mutants.

Figure 44 – Amino acid sequence alignment of heavy and light chain of the two antibodies STE90-C11 and YU536-D04 with the third antibody YU537-H11 using Clustal™ software. YU537-H11 is more distant related to STE90-C11 and YU536-D04, however, again the sequence similarity (including conservative and semi-conservative amino acid exchanges) shows again that the antibodies of the invention form a structural and functional related cluster of antibodies with outstanding performance, especially with respect to the binding to escape mutants.

Figure 45 – Binding study with STE90-C11 against the “Indian mutant” (triple mutation: L452R + E484Q + D614G). The binding profile is very similar to the binding profile of said antibody against wild-type RBD.

Figure 46 – “Chain shuffling experiment”: A hybrid-antibody comprising the heavy chain from YU536-D04 and the light chain from STE90-C11 was tested for its binding ability to wild-type RBD and different mutants. It turned out that the binding was as good as the binding with STE90-C11 (and even better in case of K417N).

Examples

Example 1: Antigen production

As antigens of the full-length SARS-CoV-2 glycoprotein S (Spike) (SEQ ID NOs: 01 & 02), full-length S1-subunit of the SARS-CoV-2 glycoprotein S (SEQ ID NOs: 03 & 04), full-length of receptor binding domain (RBD) of the S1-subunit of SARS-CoV-2 glycoprotein S (SEQ ID NOs: 05 & 06), and the extra-cellular domain of human ACE2 (full-length human ACE2: SEQ ID NOs: 07 & 08) were produced by conventional recombinant methods or ordered by external providers.

For some applications the antigens were further modified to result in active fragments or by attachment of a HIS-tag.

1.1 Design of expression vectors

Production in Expi293F cells was performed using pCSE2.5-His-XP, pCSE2.6-hFc-XP or pCSE2.6-mFc-XP (Jäger et al., 2013) where the respective single chain variable fragment of the antibodies or antigens were inserted by NcoI/NotI (NEB Biolabs) digestion. Antigen production in High Five insect cells was performed using NcoI/NotI compatible variants of the OpiE2 plasmid (Bleckmann et al., 2015) containing an N-terminal signal peptide of the mouse Ig heavy chain, the respective antigen and C-terminal either 6xHis-tag, hFc or mFc.

1.2 Production of antigens in insect cells

Different domains or subunits of the Spike protein (GenBank: MN908947) and the extracellular domain of ACE2 were Baculovirus-free produced in High Five cells (Thermo Fisher Scientific) by transient transfection as previously described in Bleckmann et al. (Bleckmann et al., 2019). Briefly, High Five cells were cultivated at 27°C, 110 rpm in ExCell405 media (Sigma) and kept at a cell density between 0.3 – 5.5 x10⁶ cells/mL. For transfection cells were centrifuged and resuspended in fresh media to a density of 4x10⁶ cells/mL and transfected with 4 µg plasmid/mL and 16 µg/mL of PEI 40 kDa (Polysciences). 4 h up to 24 h after transfection cells were fed with 75% of the transfection volume. At 48 h after transfection cell culture medium was doubled. Cell supernatant was harvested five days after transfection in a two-step-centrifugation (4 min at 180xg and 20 min at above 3500xg) and 0.2 µm filtrated for purification.

1.3 Production of antigens and scFv-Fc in mammalian cells

Antibodies, different domains or subunits of the Spike protein and the extracellular domain of ACE2 were produced in Expi293F cells (Thermo Fisher Scientific). Expi293F cells were cultured

at 37°C, 110 rpm and 5% CO₂ in Gibco FreeStyle F17 expression media (Thermo Fisher Scientific) supplemented with 8 mM Glutamine and 0.1% Pluronic F68 (PAN Biotech). At the day of transfection cell density was between 1.5 – 2 x10⁶ cells/mL and viability at least above 90%. For formation of DNA:PEI complexes 1 µg DNA/mL transfection volume and 5 µg of 40 kDa PEI (Polysciences) were first diluted separately in 5% transfection volume in supplemented F17 media. DNA and PEI was then mixed and incubated ~25 min at RT before addition to the cells. 48 h later the culture volume was doubled by feeding HyClone SFM4Transfx-293 media (GE Healthcare) supplemented with 8 mM Glutamine. Additionally, HyClone Boost 6 supplement (GE Healthcare) was added with 10% of the end volume. One week after transfection supernatant was harvested by 15 min centrifugation at 1500xg.

1.4 Protein purification

Protein purification was done depending on the production scale in 24 well filter plate with 0.5 mL resin (10 mL scale) or 1 mL column on Äkta Go (GE Healthcare), Äkta Pure (GE Healthcare) or Profina System (BIO-RAD). MabSure Select (GE Healthcare) was used as resin for Protein A purification. For His-tag purification of Expi293F supernatant HisTrap FF Crude column (GE Healthcare) and for His-tag purification of insect cell supernatant HisTrap excel column (GE Healthcare) was used. All purifications were performed according to the manufactures manual. Indicated antigens were further purified by size exclusion chromatography by a 16/600 Superdex 200 kDa pg (GE Healthcare). All antigens, antibodies and scFv-Fc were run on Superdex 200 Increase 10/300GL (GE Healthcare) on Äkta or HPLC (Techlab) on an AdvanceBio SEC 300Å 2.7 µm, 7.8x300 mm (Agilent) for quality control.

1.5 Validation of spike protein binding specifically to ACE2

ACE2 binding specifically to the produced antigens was confirmed in ELISA and on cells in cytometer.

For ELISA 200 ng ACE2-mFc per well was immobilized on a Costar High binding 96 well plate (Corning) at 4°C over night. Next, the wells were blocked with 350 µL 2% MBPST (2% (w/v) milk powder in PBS; 0.05% Tween20) for 1 h at RT and then washed 3 times with MilliQ-H₂O and 0.05% Tween20 (Tecan Hydro Speed). Afterwards, the respective antigen was added at the indicated concentrations and incubated 1 h at RT prior to another 3 times washing step. Finally, the antigen was detected using mouse-anti-polyHis conjugated with horseradish peroxidase (HRP) (A7058, Sigma) for His-tagged antigens, goat-anti-mIgG(Fc) conjugated with HRP (A0168, Sigma) for mFc tagged antigen versions or goat-anti-hIgG(Fc) conjugated with HRP (A0170, Sigma) if hFc-tagged antigens had to be detected. Bound antigens were visualized with tetramethylbenzidine (TMB) substrate (20 parts TMB solution A (30 mM Potassium citrate; 1 % (w/v) Citric acid (pH 4.1)) and 1 part TMB solution B (10 mM TMB; 10% (v/v) Acetone; 90% (v/v)

Ethanol; 80 mM H₂O₂ (30%)) were mixed). After addition of 1 N H₂SO₄ to stop the reaction, absorbance at 450 nm with a 620 nm reference was measured in an ELISA plate reader (Epoch, BioTek).

To verify the ACE2-antigen interaction on living cells, Expi293F cells were transfected according to the protocol above using pCSE2.5-ACE2fl-His and 5% eGFP plasmid. Two days after transfection purified S1-S2-His, S1-His or RBD-His was labeled using Monolith NTTM His-Tag Labeling Kit RED-tris-NTA (Nanotemper) according to the manufacturer's protocol. Fc-tagged versions were labeled indirectly by using goat-anti-mFc-APC (Dianova) or mouse anti-hFcγ₁-APC (Biolegend) antibody. 100, 50 and 25 nM of antigen were incubated with 5x10⁵ ACE2 expressing or non-transfected Expi293F cells (negative control) 50 min on ice. After two washing steps fluorescence was measured in MACSQuant Analyzer (Miltenyi Biotec.).

1.6 Production of SARS CoV2 spike domains or subunits and human ACE2 in insect cells and mammalian cells

SARS-CoV-2 RBD-SD1 (aa319-591) according to Wrapp et al. 2020 (Wrapp et al., 2020b), S1 subunit (aa14-694), S1-S2 (aa14-1210, with proline substitutions at position 986 and 987 and "GSAS" substitution at the furin site) and extracellular ACE2 were produced in insect cells using a plasmid based baculovirus free system (Bleckmann et al., 2019) as well as in Expi293F cells. All antigens with exception of S1-S2 were produced with human IgG1 Fc part, murine IgG2a Fc part or with 6xHis tag in both expression systems. S1-S2 was only produced with 6xHis tag. The extracellular domain of ACE2 was produced with human IgG1 Fc part in Expi293F cells and 6xHis tagged in insect cells. The yields of all produced proteins are given in table 3. The proteins were analyzed by size exclusion chromatography (SEC).

S1 as well as S1-S2 were produced in higher amounts in insect cells compared to Expi293F cells. RBD was produced well in both production systems. The binding of the produced spike domains/proteins to ACE2 was validated by ELISA and flow cytometry analysis on ACE2 positive cells.

Maximum production yields of SARS-CoV-2 spike protein/domains and human ACE2 in insect cells (High Five) and mammalian cells (Expi293F). Proteins with His-tag produced in High Five cells and S1-hFc* were additionally purified by SEC. * with Furin site.

Table 3: Binding Results.

	High Five cells	Expi293F cells
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	Yield	Binding specifically to ACE2 in ELISA	Binding to ACE2 on cells	Yield	Binding specifically to ACE2 in ELISA	Binding to ACE2 on cells
RBD-hFc	90 mg/L	yes	yes	203 mg/L	yes	yes
RBD-mFc	48 mg/L	yes	yes	116 mg/L	yes	yes
RBD-His	92 mg/L	yes	yes	35 mg/L	yes	yes
S1-hFc*	7 mg/L	yes	yes	<1 mg/L	no	no
S1-hFc	50 mg/L	yes	yes	<1 mg/L	weak	yes
S1-mFc	36 mg/L	yes	yes	<1 mg/L	yes	yes
S1-His	15 mg/L	yes	yes	<1 mg/L	weak	no
S1-S2-His	8 mg/L	yes	yes	<1 mg/L	no	no
ACE2-hFc	tbd	-	-	82.8 mg/L	-	-
ACE2-mFc	tbd	-	-	18.6 mg/mL	-	-
ACE-His	1 mg/L	-	-	-	-	-

Example 2: Production of therapeutic antibodies

For identification of antibodies a phage-display technique was applied. As libraries a naïve fab library as well as an immune library was used. S1-HIS was used as immobilized target. As counter selection streptavidin, IVIGs and protein N standard were used.

In total 192 clones from lambda and kappa selection were isolated. 48 sequence clusters with blocking antibodies were identified.

The number of clones was reduced for IgG conversion based on a rational approach: From each cluster at least one antibody was selected. From large clusters two clones were selected.

The following steps were performed to deselect regular antibodies, which are not suitable as therapeutic antibodies:

Step 1: Deselection of antibodies with not acceptable post-translational modifications (PTMs) (i.e. unpaired cysteines & glycosylations)

Step 2: Remaining clones were selected based on number of developability flags

Step 3: Clones with identical number of flags were selected based on ACE2:RBD inhibition

Step 4: Clones with similar ACE2:RBD inhibition were selected based on binding characteristics (i.e. RBD & S1-S2 binding was preferred over RBD binding only).

2.1 Antibody selection using phage display

The antibody selection was performed as follows: For the panning procedure, the antigen was immobilized on a Costar High binding 96 well plate (Corning). 5 µg of S1-hFc (produced in High Five cells) was diluted in carbonate puffer (50 mM NaHCO₃, pH 9.6) and coated in the wells at 4°C overnight. Next, the wells were blocked with 350 µL % MBPST (2% (w/v) milk powder in PBS; 0.05% Tween20) for 1h at RT and then washed 3 times with PBST (PBS; 0.05% Tween20).

Before adding the libraries to the coated wells, the libraries (5x10¹⁰ phage particles) were pre-incubated with 2% MPBST on blocked wells for 1h at RT. The libraries were transferred to the coated wells, incubated for 2h at RT and washed 10 times. Bound phage was eluted with 150 µL trypsin (10 µg/mL) at 37°C and was used for the next panning round.

The eluted phage solution was transferred to a 96 deep well plate (Greiner Bio-One, Frickenhausen, Germany) and incubated with 145 µL E. coli TG1 (OD₆₀₀ = 0.5) firstly for 30 min at 37°C, then 30 min at 37°C and 650 rpm to infect the phage particles. 1 mL 2xYT-GA (1.6% (w/v) Tryptone; 1 % (w/v) Yeast extract; 0.5% (w/v) NaCl (pH 7.0), 100 mM D-Glucose, 100 µg/mL ampicillin) was added and incubated for 1 h at 37°C and 650 rpm, followed by addition of 1x10¹⁰ cfu M13KO7 helper phage.

Subsequently, the infected bacteria were incubated for 30 min at 37°C followed by 30 min at 37°C and 650 rpm before centrifugation for 10 min at 3220xg.

The supernatant was discarded and the pellet resuspended in fresh 2xYT-AK (1.6% (w/v) Tryptone; 1 % (w/v) Yeast extract; 0.5% (w/v) NaCl (pH 7.0), 100 µg/mL ampicillin, 50 µg/mL kanamycin). The phage antibodies were amplified overnight at 30°C and 650 rpm and used for the next panning round. In total four panning rounds were performed. In each round, the stringency of the washing procedures was increased (20x in panning round 2, 30x in panning round 3, 40x in panning round 4) and the amount of antigen was reduced (2.5 µg in panning round 2, 1.5 µg in panning round 3 and 1 µg in panning round 4). After the fourth, respectively third panning round, the titer plate was used to select monoclonal antibody clones for the screening ELISA or for batch cloning to pCSE2.6-hlgG1-Fc-XP.

Antibody selection strategies using the human naive antibody gene libraries HAL9/10. n.a. = not applicable.

Antibody selection campaign	library	target	panning rounds	binders/ screened clones	unique antibodies	cloned as scFv-Fc	inhibiting antibodies
STE70	HAL9/10	S1-hFc (Hi5)	4	7/ 94	7	7	1
STE72	HAL10 (kappa)	S1-hFc (Hi5)	4	397/ 752	90	44	8
STE73	HAL9 (lambda)	S1-hFc (Hi5)	4	519/ 846	209	59	8
STE77	HAL10 (kappa)	S1-hFc (Expi)	4	7/ 564	2	n.a.	n.a.
STE78	HAL9 (lambda)	S1-hFc (Expi)	4	10/ 282	1	n.a.	n.a.

2.2 Screening of monoclonal recombinant binders using E. coli scFv supernatant

Soluble antibody fragments (scFv) were produced in 96-well MTPs with polypropylene (U96 PP, Greiner Bio-One). 150 µL 2xYT-GA was inoculated with the bacteria bearing scFv expressing phagemids. MTPs were incubated overnight at 37°C and 800 rpm in a MTP shaker (Thermoshaker PST-60HL-4, Lab4You, Berlin, Germany). A volume of 140 µL 2xYT-GA in a PP-MTP well was inoculated with 10 µL of the overnight culture and grown at 37°C and 800 rpm until bacteria reached an OD₆₀₀ of 0.5. Bacteria were harvested by centrifugation for 10 min at 3220xg and the supernatant was discarded. To induce expression of the antibody genes, the pellets were resuspended in 150 µL 2xYT supplemented with 100 µg/mL ampicillin and 50 µM isopropyl-beta D thiogalactopyranoside (IPTG) and incubated at 30°C and 800 rpm overnight.

Bacteria were pelleted by centrifugation for 10 min at 3220xg and 4°C. The scFv-containing supernatant was transferred to a new PP-MTP and stored at 4°C before ELISA analysis.

For the ELISA, 100 ng of antigen was coated on 96 well microtiter plates (MaxiSorp, Thermo Fisher Scientific) in PBS (pH 7.4) overnight at 4°C. After coating, the wells were washed three times with PBST and blocked with 2% MPBST for 1 h at RT, followed by three washing steps with PBST. Supernatants containing monoclonal scFv were mixed with 2% MPBST (1:2) and incubated in the antigen coated plates for 1.5 h at RT followed by three PBST washing cycles. Bound scFv were detected using murine mAb 9E10 which recognizes the C-terminal c-myc tag (1:50 diluted in 2% MPBST) and a goat anti-mouse serum conjugated with horseradish peroxidase (HRP) (A0168, Sigma) (1:50,000 dilution in 2% MPBST).

Bound antibodies were visualized with tetramethylbenzidine (TMB) substrate (20 parts TMB solution A (30 mM Potassium citrate; 1% (w/v) Citric acid (pH 4.1)) and 1 part TMB solution B (10 mM TMB; 10% (v/v) Acetone; 90% (v/v) Ethanol; 80 mM H₂O₂ (30%)) were mixed). After stopping the reaction by addition of 1 N H₂SO₄, absorbance at 450 nm with a 620 nm reference was measured in an ELISA plate reader (Epoch, BioTek). Monoclonal binders were sequenced and analyzed using VBASE2 (www.vbase2.org) (Mollova et al., 2010).

2.3 Inhibition of S1-S2 binding specifically to ACE2 expressing cells using MacsQuant

The inhibition tests in cytometer on EXPI293F cells were performed based on the protocol for validation of spike protein binding specifically to ACE2 (see above) but only binding specifically to S1-S2-His antigen (High Five cell produced) was analysed. The assay was done in two set-ups. In the first setup 50 nM antigen was incubated with min. 1 µM of different scFv-Fc and the ACE2 expressing cells. The resulting median antigen fluorescence of GFP positive living single cells was measured. For comparison of the different scFv-Fc first the median fluorescence background of cells without antigen was subtracted, second it was normalized to the antigen signal where no antibody was applied. All scFv-Fc showing an inhibition in this setup were further analysed by titration (max. 1500 nM- 4.7 nM) on S1-S2-His (High Five cell produced). The IC₅₀ was calculated using the equation $f(x) = A_{min} + (A_{max} - A_{min}) / (1 + (x_0/x)^h)^s$ and parameters from Origin. In addition, pairwise combinations (max. 750 nM of each scFv-Fc) of the different inhibiting scFv-Fc were tested.

2.4 Characterization of the scFv-Fc in titration ELISA

The ELISA were performed as described above in "Screening of monoclonal recombinant binders using E.coli scFv supernatant". For titration ELISA the purified scFv-hFc were titrated from 10 µg/mL- 0.001 µg/mL on S1-S2-His (High Five cell produced), RBD-mFc (High Five cell produced), S1-mFc (High Five cell produced). In addition, all 10µg/µL scFv-hFc were also tested

for cross reactivity on Expi293F cell lysate, BSA and lysozyme. The scFv-hFc were detected using goat-anti-hlgG(Fc)-HRP (A0170, Sigma). Titration assays were performed using 384 well microtiter plates using Precision XS microplate sample processor (BioTek), EL406 washer dispenser (BioTek) and BioStack Microplate stacker (BioTek). EC50 were calculated with by GraphPad Prism Version 6.1) fitting to a four-parameter logistic curve).

2.5 Selection of antibodies by phage display

Antibodies were selected against S1 in four panning rounds in microtiter plates. The complete S1 subunit was used for antibody generation to ensure a native conformation of the RBD. The antibody screening by antigen ELISA was performed with soluble monoclonal scFv produced in E. coli in 96 well MTPs. Subsequently, binders were DNA sequenced and unique antibodies were recloned as scFv-Fc. Three pannings were performed. In a first approach the lambda (HAL9) and kappa (HAL10 library) were combined and the antigen S1-hFc (with Furin site, produced in High Five cells) was immobilized in PBS.

In the second approach, the pannings were performed separately for HAL10 and HAL9 using S1-hFc (with Furin site, produced in High Five cells) immobilized in carbonate buffer. Here, unique antibodies were selected from HAL10 and from HAL9.

In a third approach, S1-hFc produced in Expi293F cells was used. Here, the panning resulted in only three unique antibodies and these antibodies were not further analyzed in inhibition assays.

The Antibody subfamily distribution was analyzed and compared to the subfamily distribution in the HAL9/10 library and in vivo. Antibodies were selected covering the main gene VH gene families VH1 and VH3 whereas only few antibodies were selected using VH4. From the selected antibodies the V-gene VH3-23 was selected 96x. Therefore, this V-gene was used in 30% of the antibodies. In case of the lambda, the V-gene distribution is similar the distribution in the library. Here, it's only the V-gene VL6-57. For kappa, VK2 and VK4 are underrepresented compared to the library.

2.6 Transient production of anti-SARS-CoV-2 scFv-Fc in mammalian cells

Because of throughput issues only a selection of the unique antibodies was chosen for production. In addition, antibodies with glycosylation sites in the CDRs were excluded from the production as scFv-Fc because of developability issues. From all antibodies some antibodies were further produced as scFv-Fc in 5 mL scale. The production yields were in the range of 20-440 mg/L.

2.7 Antibodies inhibit the binding of S1 to ACE2 positive cells

The inhibition was measured by flow cytometry using ACE2 positive cells and labelled S1-S2 trimer. The spike protein was used for this inhibition assay to mimic the viral binding specifically to ACE2 positive cells. In a first screening step, all cloned antibodies were measured with 1500 nM antibodies (molar ratio antibody: S1-S2 30:1) and antibodies with minimum ~75% inhibition were selected for further analysis.

In a next step, the inhibiting antibodies were further tested in different concentrations from 1500 nM to 4.7 nM (molar ratio 30:1 to ~1:10) in the cell based flow cytometry assay. The results of these inhibitions assays are given in figure 2B and table 4. The antibodies showed 50% inhibition with molar ratios of antibody binding site (antibody arm) to spike monomer of 0.8:1 to 13:1. The best antibodies showed 50% inhibition with a molar ratio of 0.8 antibody arms to 1 spike (monomer). To validate these results, we performed the inhibition ELISA also on RBD in the cell based flow cytometry assay because RBD-mFc can be used in a lower amount of 10 nM in this assay (Figure 2C). With the exception of two antibodies all antibodies show a good inhibition of RBD with best molar ratios of 0.3-0.6.

The inhibition of the antibodies was validated on human Calu-3 cells which are ACE2 positive using RBD and S1-S2 showing a stronger inhibition on Calu-3 compared to the transiently over-expressing ACE2 positive Exp293Fi cells. The Expi293F system allowed the better discrimination between the degree of inhibition when using the complete S1-S2 spike protein, because the S1-S2 is directly labelled with a fluorophore and the signals are not amplified in comparison to RBD with a murine Fc and a secondary fluorophore labelled antibody. This assay also showed, that all inhibiting antibodies selected against S1 are binding RBD.

2.8 Determination of the EC50 of the inhibiting antibodies

The EC50 was measured by titration ELISA for the inhibiting antibodies on RBD, S1 and S1-S2 spike (figure 3). It was shown, that all inhibiting antibodies bound RBD. The best antibodies showed a half maximal binding at 0.7 nM, respectively 0.6 nM, on RBD. The EC50 values were slightly different between binding specifically to RBD or S1 subunit. The EC50 on S1-S2 spike was reduced for some of the antibodies compared to RBD or S1.

2.9 Synergistic effects of antibody combinations

The best antibodies were tested in the flow cytometry inhibition assay in pairwise combinations using 1500 nM antibody and 50 nM spike. Here, the combinations showed an improved inhibition compared to the single antibodies. The most antibody combinations were chosen using a lineage tree analysis (Genious Tree Builder) to reduce the amount of combinations to be tested.

The following therapeutic antibodies as depicted in SEQ ID NOs 9 to 1288 were finally identified. In another round of experiments the further antibodies as depicted in SEQ ID NOs 1289 to 1728 were finally identified.

For reference please be referred to table 4, which lists sequences segment (e.g. CDR, VL, etc.), sequence type (DNA or Protein "PRT"), antibody-Name and SEQ ID NO of the first three antibodies.

Table 4: Reference of antibodies and SEQ ID NOs

Sequence Segment	Type	Antibody-Name	SEQ ID NO.	Antibody-Name	SEQ ID NO.	Antibody-Name	SEQ ID NO.
CDR1 of heavy chain (H-CDR1)	DNA	YU535-C09	9	YU537-B05	29	YU534-H01	49
CDR1 of heavy chain (H-CDR1)	PRT	YU535-C09	10	YU537-B05	30	YU534-H01	50
CDR2 of heavy chain (H-CDR2)	DNA	YU535-C09	11	YU537-B05	31	YU534-H01	51
CDR2 of heavy chain (H-CDR2)	PRT	YU535-C09	12	YU537-B05	32	YU534-H01	52
CDR3 of heavy chain (H-CDR3)	DNA	YU535-C09	13	YU537-B05	33	YU534-H01	53
CDR3 of heavy chain (H-CDR3)	PRT	YU535-C09	14	YU537-B05	34	YU534-H01	54
CDR1 of light chain (L-CDR1)	DNA	YU535-C09	15	YU537-B05	35	YU534-H01	55
CDR1 of light chain (L-CDR1)	PRT	YU535-C09	16	YU537-B05	36	YU534-H01	56

CDR2 of light chain (L-CDR2)	DNA	YU535-C09	17	YU537-B05	37	YU534-H01	57
CDR2 of light chain (L-CDR2)	PRT	YU535-C09	18	YU537-B05	38	YU534-H01	58
CDR3 of light chain (L-CDR3)	DNA	YU535-C09	19	YU537-B05	39	YU534-H01	59
CDR3 of light chain (L-CDR3)	PRT	YU535-C09	20	YU537-B05	40	YU534-H01	60
variable light chain (VL)	DNA	YU535-C09	21	YU537-B05	41	YU534-H01	61
variable light chain (VL)	PRT	YU535-C09	22	YU537-B05	42	YU534-H01	62
variable heavy chain (VH)	DNA	YU535-C09	23	YU537-B05	43	YU534-H01	63
variable heavy chain (VH)	PRT	YU535-C09	24	YU537-B05	44	YU534-H01	64
complete light chain (L)	DNA	YU535-C09	25	YU537-B05	45	YU534-H01	65
complete light chain (L)	PRT	YU535-C09	26	YU537-B05	46	YU534-H01	66
complete heavy chain (H)	DNA	YU535-C09	27	YU537-B05	47	YU534-H01	67
complete heavy chain (H)	PRT	YU535-C09	28	YU537-B05	48	YU534-H01	68

Corresponding to the same consecutive pattern outlined in table 4, the further antibodies correspond to the following SEQ ID NOs as outlined in tables 5 to 7:

Table 5: Further references of antibodies and corresponding SEQ ID NOs

Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs
YU534-F11	69 - 88	YU534-D06	269 - 288	YU534-F06	469 - 488
YU536-C01	89 - 108	YU536-E07	289 - 308	YU534-C05	489 - 508
YU535-A06	109 - 128	YU534-B03	309 - 328	YU534-D09	509 - 528
YU535-D12	129 - 148	YU534-D10	329 - 348	YU535-C06	529 - 548
YU534-C09	149 - 168	YU536-D04	349 - 368	YU535-A09	549 - 568
YU534-C12	169 - 188	YU537-B08	369 - 388	YU535-G09	569 - 588
YU535-A02	189 - 208	YU537-H11	389 - 408	YU536-H01	589 - 608
YU535-E07	209 - 228	YU534-A11	409 - 428	YU536-H05	609 - 628
YU536-E12	229 - 248	YU534-G06	429 - 448	YU535-G02	629 - 648
YU537-E03	249 - 268	YU534-H02	449 - 468	YU535-G11	649 - 668

Table 6: Further references of antibodies and corresponding SEQ ID NOs

Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs
YU534-B11	669 - 688	YU534-G10	869 - 888	YU505-E02	1069 - 1088
YU534-C10	689 - 708	YU505-A01	889 - 908	YU505-E04	1089 - 1108

YU535-D10	709 - 728	YU505-A02	909 - 928	YU505-F03	1109 - 1128
YU535-B02	729 - 748	YU505-A05	929 - 948	YU505-F04	1129 - 1148
YU534-D07	749 - 768	YU505-B03	949 - 968	YU505-F05	1149 - 1168
YU537-H08	769 - 788	YU505-B04	969 - 988	YU519-A01	1169 - 1188
YU534-E01	789 - 808	YU505-B06	989 - 1008	YU519-A09	1189 - 1208
YU534-C02	809 - 828	YU505-D01	1009 - 1028	YU519-B05	1209 - 1228
YU534-C06	829 - 848	YU505-D02	1029 - 1048	YU519-C01	1229 - 1248
YU536-C12	849 - 868	YU505-E01	1049 - 1068	YU519-E03	1249 - 1268
				YU519-E12	1269 - 1288

Table 7: Further references of antibodies and corresponding SEQ ID NOs

Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs	Antibody Name	SEQ ID NOs
PHE162-D2	1289-1296	STE91-B1-B3	1473-1480	STE72-2G4	1657-1664
STE90-A10	1297-1304	STE94-A1	1481-1488	STE73-2B2	1665-1672
STE90-C11	1305-1312	STE94-B2	1489-1496	STE73-2C2	1673-1680
STE90-D7	1313-1320	STE94-D3	1497-1504	STE73-2E9	1681-1688
STE90-G2	1321-1328	STE94-E3	1505-1512	STE73-2G8	1689-1696
STE90-B1-A10	1329-1336	STE94-E9	1513-1520	STE73-6B10	1697-1704

STE90-B1-B4	1337-1344	STE94-F6	1521-1528	STE73-6C1	1705-1712
STE90-B1-D1	1345-1352	STE94-F7	1529-1536	STE73-6C8	1713-1720
STE90-B1-D6	1353-1360	STE94-F12	1537-1544	STE73-9G3	1721-1728
STE90-B1-F8	1361-1368	STE94-H2	1545-1552		
STE90-B2-A1	1369-1376	STE94-B1-D1	1553-1560		
STE90-B2-B3	1377-1384	STE94-B1-E12	1561-1568		
STE90-B2-C5	1385-1392	STE94-B2-B1	1569-1576		
STE90-B2-D12	1393-1400	STE94-B2-B6	1577-1584		
STE90-B2-E6	1401-1408	STE94-B2-E11	1585-1592		
STE90-B3-B10	1409-1416	STE70-1E12	1593-1600		
STE90-B3-H3	1417-1424	STE72-1B6	1601-1608		
STE90-B3-H6	1425-1432	STE72-1G5	1609-1616		

STE91-C4	1433-1440	STE72-4C10	1617-1624		
STE91-D4	1441-1448	STE72-4E12	1625-1632		
STE91-F6	1449-1456	STE72-8A2	1633-1640		
STE91-F10	1457-1464	STE72-8A6	1641-1648		
STE91-B1-A10	1465-1472	STE72-8E1	1649-1656		

In one embodiment from these antibodies YU505-A01 (SEQ ID NOs: 889 – 908), YU505-A02 (SEQ ID NOs: 909 - 928), YU505-E01 (SEQ ID NOs: 1049 – 1068) and YU505-F05 (SEQ ID NOs: 1149 - 1168) have been selected for further characterization.

In a further embodiment from these antibodies YU534-C09 (SEQ ID Nos: 149 – 168), YU534-C12 (SEQ ID NOs: 169 - 188), YU534-D09 (SEQ ID NOs: 509 - 528), YU535-A02 (SEQ ID NOs: 189 - 208), YU536-D04 (SEQ ID NOs: 349 - 368), YU537-H08 (SEQ ID NOs: 769 - 788) and YU537-H11 (SEQ ID NOs: 389 - 408) have been selected for further characterization.

The CDR-sequences of these four antibodies are also depicted in Figure 3, their VL and VH-sequences are depicted in Figures 4a to 4d. The scFv DNA-sequences of fragments of these antibodies are depicted in Figure 5a to 5d.

Example 3: Binding and Competition Assays

Different binding characteristics of the four antibodies were analyzed.

Name	Specificity	ACE2:RBD-competition at [5 µg/ml antibody]	ACE2:RBD-competition at [0.5 µg/ml antibody]	K _D
YU505-A01	RBD binding	less than 30% of ACE2 bound to RBD	less than 60% of ACE2 bound to RBD	3.41 x 10 ⁻⁹
YU505-A02	RBD binding	less than 30% of	less than 60% of	1.07 x 10 ⁻⁸

		ACE2 bound to RBD	ACE2 bound to RBD	
YU505-E01	RBD binding	less than 25% of ACE2 bound to RBD	less than 50% of ACE2 bound to RBD	4.84×10^{-9}
YU505-F05	RBD + S1 binding	less than 30% of ACE2 bound to RBD	less than 60% of ACE2 bound to RBD	1.31×10^{-8}

Example 4: Infectious SARS-CoV-2 neutralizing assay (Confluent VERO E6-Test)

Antibodies were diluted in culture medium and mixed with 50 μ l (500 TCID₅₀) SARS-CoV-2 for 1 hour. The mixture was then added to VERO E6 cells. As controls cells were grown either with medium only or with the SARS-CoV-2 mixture without adding any antibody. Cells were incubated for 1 hour, after which the cells were washed and further incubated in medium for 3d and 18h.

In another embodiment antibodies were diluted in 1:10-steps and mixed with 5 μ l (50 TCID₅₀) SARS-CoV-2-virus. The mix is then added to the cells. Cells are washed after 1h and incubated with methyl-cellulose-containing medium. After 3 days single plaques become visible within the cell layer, which can be counted.

Confluence of cells was measured at 4 dpi (cf. Figure 2).

Table 8: Result of Infectious SARS-CoV-2 neutralizing assay (Confluent VERO E6-Test)

Antibody	Confluence Score in VERO E6 cell assay
YU505-A01	> 99%
YU505-A02	> 99%
YU505-E01	> 98%
YU505-F05	> 95%

Example 5: SEC-HPLC of specific Ab

Size-exclusion HPLC was performed with the four antibodies YU505-A01, YU505-A02, YU505-E01 and YU505-F05.

YU505-A01 and YU505-E01 showed no, YU505-A02 and YU505-F05 showed only a very small agglutination-peak (the peaks at time-points > 10 are contaminations of the column), confirming the usability of the antibodies for therapeutic application.

See also Figure 7.

Example 6: Specificity ELISA

In a specificity ELISA the “stickiness” of some antibodies was compared with Avelumab-control.

Mean S/N stickiness (test antibodies vs. Avelumab) was below 1 for YU505-A01 (about 0.5), YU505-A02 (about 0.6) and YU505-E01 (about 0.8).

See table 9 and Figure 6.

Table 9: Results of Specificity ELISA

	YU505-F05	YU505-E01	YU505-A01	YU505-A02	Trastuzumab	Trastuzumab (silenced Fc)	Palivizumab	Palivizumab (silenced Fc)	IVIGs	Avelumab	2% BSA-T
DNA	0.3	0.3	0.2	0.3	0.4	0.6	0.3	0.5	0.6	1.0	0.1
LPS	1.3	1.1	0.9	0.8	1.5	1.2	1.3	1.2	3.9	1.0	0.8
Lysozyme	1.1	1.4	0.7	0.9	1.0	0.6	0.8	0.9	0.7	1.0	0.3
Cell lysate	1.7	0.4	0.2	0.2	0.3	0.2	0.3	0.3	0.3	1.0	0.1
Mean stickiness	1.1	0.8	0.5	0.6	0.8	0.7	0.7	0.7	1.4	1.0	0.3

Stdev. stickyness	0.6	0.5	0.4	0.4	0.5	0.4	0.5	0.4	1.7	0.0	.03
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Please note: Values >1.0 indicate increased “stickyness” compared to Avelumab

Example 7: Melting Temperature Analysis

Antibodies were analyzed by nanoDSF which uses tryptophan or tyrosin fluorescence to monitor protein unfolding in terms of effect on the onset melting temperature ($T_{m \text{ onset}}$), the melting temperature (T_m) and aggregation temperature (T_{agg}) of the target protein.

10 Antibodies were tested, the target assay concentration was 0.15 mg/ml, the assay buffer was 1x PBS pH 7.4, assay samples were prepared by diluting the target protein from its stock to the given concentration in assay buffer, all samples were stored at -80°C prior to the experiment phase.

The experiments were performed on a Prometheus NT.48™ device equipped with additional back reflection options for detection of target protein aggregation. The technical parameters were as follows: The LED-sensitivity was set at 50% (the capillary scan indicated that 50% LED intensity resulted in sufficient intrinsic fluorescence of the targets), the temperature range was 20-95°C, the heating speed 1°C/min. The capillary type was a “high sensitivity”-type. The ratio 350/330 nm was used for protein unfolding (T_m) and onset of protein unfolding ($T_{m \text{ onset}}$). Back scattering for sample aggregation (T_{agg}).

All samples showed comparable fluorescence signals. No protein aggregation was observed at the starting temperature of 20°C, indicated by scattering signals (crosses) around 80 mAU.

Measurements were done in duplicates and the mean as well as the error deviation was calculated. Data were analyzed using the PR.ThermalControl™ v2.0.4 software from Nanotemper Technologies™.

The results of the analysis are summarized in Table 10.

Table 10: Summary of results of T_m onset, T_{m1} , T_{m2} , and T_{agg} for the tested antibodies:

Sample	T_m onset	T_{m1}	T_{m2}	T_{agg}
YU505-A01	$61.6 \pm 0.1^\circ\text{C}$	$68.8 \pm 0.1^\circ\text{C}$	$81.9 \pm 0.1^\circ\text{C}$	$71.3 \pm 0.1^\circ\text{C}$
YU505-A02	$60.1 \pm 0.2^\circ\text{C}$	$70.0 \pm 0.2^\circ\text{C}$	$79.7 \pm 0.1^\circ\text{C}$	$78.6 \pm 0.2^\circ\text{C}$
YU505-E01	$57.1 \pm 0.3^\circ\text{C}$	$60.3 \pm 0.0^\circ\text{C}$	$68.2 \pm 0.1^\circ\text{C}$	$68.4 \pm 0.1^\circ\text{C}$
YU505-F05	$57.3 \pm 0.3^\circ\text{C}$	$64.5 \pm 0.0^\circ\text{C}$	$81.6 \pm 0.1^\circ\text{C}$	$69.5 \pm 0.3^\circ\text{C}$
YU534-C12	$65.3 \pm 0.8^\circ\text{C}$	$69.5 \pm 0.0^\circ\text{C}$	$81.4 \pm 0.7^\circ\text{C}$	$71.2 \pm 0.0^\circ\text{C}$
YU534-D09	$62.4 \pm 0.1^\circ\text{C}$	$68.9 \pm 0.0^\circ\text{C}$	$81.4 \pm 0.1^\circ\text{C}$	$69.9 \pm 0.0^\circ\text{C}$
YU535-A02	$62.2 \pm 0.1^\circ\text{C}$	$70.8 \pm 0.2^\circ\text{C}$	$81.9 \pm 0.2^\circ\text{C}$	$75.9 \pm 0.0^\circ\text{C}$
YU536-D04	$62.4 \pm 0.0^\circ\text{C}$	$69.3 \pm 0.1^\circ\text{C}$	$80.9 \pm 0.0^\circ\text{C}$	$81.2 \pm 0.3^\circ\text{C}$
YU537-H08	$56.9 \pm 0.2^\circ\text{C}$	$62.1 \pm 0.2^\circ\text{C}$	$68.0 \pm 0.2^\circ\text{C}$	$70.1 \pm 0.1^\circ\text{C}$
YU537-H11	$63.7 \pm 0.0^\circ\text{C}$	$70.8 \pm 0.2^\circ\text{C}$	—	$71.7 \pm 0.2^\circ\text{C}$

Example 8: Further characteristics of the antibodies

Antibodies were further characterized regarding ACE2:RBD-inhibition, isoelectric point (pI) of human IgG, hydrophobicity and charge. The results are outlined in table 11.

Table 11: Further characteristics of the antibodies

Antibody	ACE2:RBD inhibition with 5 µg/ml IgG [%]	pI human IgG	Germinality Index VH [%]	Germinality Index VL [%]	Total hydrophobicity score	CDR hydrophobicity score	CDR charge total
YU535-C09		8.11	91.9	96.9	4.09	1.28	-1.03
YU537-B05		8.3	99.1	100	4	1.94	-0.49
YU534-H01		8.21	99.1	99	3.26	1.23	-0.6
YU534-F11		8.01	96.3	97	3.7	1.6	0.15
YU536-C01		8.11	98.2	99	3.89	1.23	-1.36
YU535-A06		8.11	97.2	100	2.73	0.45	-0.63
YU535-D12		8.21	97.2	99	3.22	0.58	-0.49
YU534-C09		8.11	84.4	100	5.08	2.09	-0.83
YU534-C12		8.21	99.1	96	4.36	1.84	0.3
YU535-A02		8.3	99.1	98	2.82	0.46	-0.7
YU535-E07		8.3	97.2	99	2.96	0.79	-0.6
YU536-E12		7.74	97.2	100	3.9	0.47	-1.45
YU537-E03		7.88	96.3	99	3.9	0.75	-0.89
YU534-D06		7.88	97.2	100	3.56	1.47	-1.71
YU536-E07		8.11	98.1	100	4.31	1.17	-0.1
YU534-B03		7.74	97.2	99	4.3	0.87	-1.24
YU534-D10		8	98.1	99	4.52	1.3	-0.14
YU536-D04		8.39	95.4	96.9	4.37	1.45	-0.25
YU537-B08		8.11	97.2	97	4.43	2.05	-0.47
YU537-H11		8.11	96.3	99	4.64	1.29	-0.19
YU534-A11		8.21	99.1	100	4.38	1.71	-0.86
YU534-G06		8.11	94.5	95	3.83	1.52	-0.52
YU534-H02		7.58	93.6	96	3.38	1.55	0.53
YU534-F06		8.01	93.6	98	4.3	2.2	0.07
YU534-C05		8.31	93.6	93	4.54	1.53	-0.09

YU534-D09		8.11	95.4	99	3.65	1.58	0.01
YU535-C06		8.21	99.1	99	2.57	0.49	-1.18
YU535-A09		7.88	100	95.9	3.27	0.52	-1.7
YU535-G09		8	100	100	3.17	0.48	-0.72
YU536-H01		8	100	99	3.31	0.76	-0.54
YU536-H05		7.87	99.1	98	2.93	0.75	-0.7
YU535-G02		8	99.1	91.8	2.98	0.45	-1.05
YU535-G11		8	100	96.9	3.43	0.75	-0.87
YU534-B11		7.88	98.2	100	4.25	0.77	-1.27
YU534-C10		8	97.2	100	4.45	0.95	-1.81
YU535-D10		8.11	98.2	98	3	0.77	-1.2
YU535-B02		7.87	99.1	91.8	2.41	0.59	-1.56
YU534-D07		8	100	99	3.09	0.6	-2.17
YU537-H08		8.01	98.2	97.9	-	-	-
YU534-E01		7.88	93.6	93.9	-	-	-
YU534-C02		7.88	96.3	91	4.69	1.55	-2.92
YU534-C06		7.74	96.3	93	4.38	1.02	-1.52
YU536-C12		7.75	96.3	94	3.92	1.45	-1.58
YU534-G10		8	95.4	98	3.91	1.46	-1.22
YU505-A01	102.2	8.11	100	98	3.41	0.5	-0.58
YU505-A02	43.8	7.75	99.1	100	6.02	3.14	-0.63
YU505-A05	52.7	8.11	100	92	3.84	1.76	0.09
YU505-B03	78.9	8.56	98.2	93.9	3.13	0.53	0.82
YU505-B04	76.6	8.01	100	99	4.22	0.88	-0.32
YU505-B06	58.5	8.12	98.2	100	4.29	0.62	-1.28
YU505-D01	89.0	8.39	100	97	3.12	0.35	-1.49
YU505-D02	49.4	8.12	100	99	4.62	1.16	-0.55
YU505-E01	24.0	7.88	93.6	97	5.18	1.86	-0.85
YU505-E02	58.8	8.21	100	97	6.46	3.11	0.24
YU505-E04	28.0	7.74	100	96	4.57	1.72	-1.22
YU505-F03	42.2	8.3	96.3	96	3.14	0.48	-1.13
YU505-F04	79.1	7.74	100	92	4.46	1.37	-1.15
YU505-F05	26.7	8.31	93.6	95.9	4.5	0.44	-0.48

YU519-A01	74.1	7.88	98.2	92.9	5.22	2.65	-1.97
YU519-A09	35.6	7.88	97.2	94.8	4.63	1.17	-2.46
YU519-B05	128.4	8.21	100	99	5.08	2.71	0.53
YU519-C01	140.7	8.48	98.2	98	3.9	1.11	-0.57
YU519-E03	148.9	8.11	100	98	4.23	1.82	0.63
YU519-E12	104.1	8	100	94.9	4.84	2.21	0.08

Example 9: IC₅₀-measurements in Vero E6 Cells

Screening and titration of some therapeutic monoclonal antibodies for SARS-CoV-2 neutralization in cell culture VeroE6 cells (ATCC CRL-1586) were seeded at a density of 6×10^4 /well onto cell culture 96-well plates (Nunc, Cat.#167008). Two days later, cells reached 100% confluence. For neutralization screening, antibodies (1 µg/ml final concentration) were mixed with the virus inoculum (250 pfu/well), using strain SARS-CoV-2/Münster/FI 110320/1/2020, in 100 µl full VeroE6 culture medium (DMEM, 10% FCS, 2 mM glutamine, penicillin, streptomycin) in technical quadruplicates or six fold replicates and incubated for 1 hour at 37°C.

Then, cells were overlaid with the antibody/virus mix and phase contrast images were taken automatically using a Sartorius IncuCyte S3 (10x objective, two hours image intervals, 4 images per well) housed in a HeraCell 150i incubator (37°C, 100% humidity, 5% CO₂).

Image data was quantified with the IncuCyte S3 GUI tools measuring the decrease of confluence concomitant with the cytopathic effect of the virus in relation to uninfected controls and controls without antibody and analyzed with GraphPad Prism 8.

For antibody titration, serial dilutions of antibodies from 1 µg/ml to 0.3 ng/ml were incubated with 25 pfu/well for one hour at 37°C and then added to confluent VeroE6 cells on 96-well cell culture plates for one hour in the incubator (100 µl/well).

Finally, the inoculum was removed and cells were overlaid with 100 µl MEM containing 1.5% methyl-cellulose.

Three days post infection, resulting plaques per well were counted from phase contrast images obtained with a Sartorius IncuCyte S3.

IC₅₀ was determined by fitting a sigmoidal function (four parameter logistic growth) using GraphPad Prism 8™.

Further results are listed in Figure 17.

Example 10: Features of the most preferred antibodies

Features of the most preferred antibodies are depicted in tables 12 to 29.

Table 12.

mAb	Origin	Germline VH	Germline VL	Virus Neutralization at 1 µg/mL	
				IgG [mean %]	scFv-Fc [median %]
STE90-C11	immune scFv library	IGHV3-66*04	IGKV1-9*01	n.d.	100
YU536-D04	immune scFv library	IGHV3-66*02	IGKV1-9*01	99	n.d.
YU537-H11	immune scFv library	IGHV3-53*01	IGLV1-51*01	93	n.d.

Table 13.

mAb	Virus neutralization (96-well titration)	Virus neutralization (24-well titration)	cell based ACE2:10 nM RBD inhibition (FACS)
	TCID50 (IgG) [nM]	TCID50 (IgG) [nM]	scFv-Fc IC50[nM]
STE90-C11	0.91	0.56	n.d.
YU536-D04	0.20	0.40	n.d.
YU537-H11	0.27	0.47	n.d.

Table 14.

mAb	cell based ACE2:50 nM S1-S2 inhibition (FACS)		ACE2:RBD Competition ELISA
	IgG IC50[nM]	scFv-Fc IC50[nM]	RBD binding activity at 33 nM IgG (=5 µg/ml) [%]
STE90-C11	13	n.d.	n.d.
YU536-D04	n.d.	n.d.	6
YU537-H11	n.d.	n.d.	10

Table 15.

mAb	ELISA EC50 (IgG)		ELISA EC50 (scFv-Fc)			
	RBD [nM]	S1-S2 [nM]	S1 [nM]	RBD [nM]	S1-S2 [nM]	S1 [nM]
STE90-C11	0.3	0.5	0.3	n.d.	n.d.	n.d.
YU536-D04	1.1	n.d.	n.d.	n.d.	n.d.	n.d.
YU537-H11	1.4	n.d.	n.d.	n.d.	n.d.	n.d.

Table 16.

mAb	Epitope	Yield (Expi293F) IgG [mg/L]	SDS- PAGE	High MW fraction in SEC [%]			Melt. Temp [°C]	Agg. Temp [°C]
				normal	24 h at pH3	24 h at 45°C		
STE90-C11	n.d.	59	ok	0.0	n.d.	0.0	n.d.	n.d.
YU536-D04	confor- matio- nal	n.d.	ok	0.0	1.1	0.0	69	81
YU537-H11	confor- matio- nal	n.d.	ok	0.0	0.0	0.0	71	72

Table 17.

mAb	S/N sticki- ness to DNA	S/N sticki- ness to LPS	S/N stickiness to Lysozyme	S/N stickiness to cell ly- sate
STE90-C11	1.0	1.0	1.0	0.7
YU536-D04	0.3	1.2	0.6	0.3
YU537-H11	0.3	1.2	0.7	0.6

Table 18.

mAb	CDR of VH	CDR of VL

STE90-C11	GLTVSSNY_IYSGGST_ARDVADAFDI	QGISSY_AAS_QQLN-SYPPFT
YU536-D04	SNYMS_VIYSGGSTYYADSVKG_DLVVM-GLDV	RASQGISSYLA_AAST-LQS_QQLNSYPIT
YU537-H11	SNYMS_VIYSGGSTYYADSVKG_GES-GSPYGM DV	SGSSSNIGN-NYVS_DTNKRPS_ATWDS-SLSVGV

Table 19.

mAb	AA of VH
STE90-C11	QVQLVESGGGLVQPGGSLRLSCAASGLTVSSNYMSWVRQAPGK- GLEWWSV/IYSGGSTYYADSVKGRFTISRDDSKNTLYLQMNSLRAEDTA- VYYCARDVADAFDIWGQGTMTVTVSS
YU536-D04	QVQLVQSGGGLVQPGGSLRLSCAASGLTVSSNYMSWVRQAPGK- GLEWWSVIYSGGSTYYADSVKGRFIISRDN SKNTLYLQMNSLRAEDTA- VYYCARDLVVMGLDVWGKGLTVTVSS
YU537-H11	EVQLLES GGGLIQPGGSLRLSCAASGLTVSSNYMSWVRQAPGK- GLEWWSVIYSGGSTYYADS- VKGRFTISRDN SKNALYLQMNSLRAEDTAVYYCARGESGSPYGM DVWGQGT LVT VSS

Table 20.

mAb	AA of VL
STE90-C11	DIVMTQSPSFLSASVGDRTITCRASQGISSYLA WYQQKPGKAPKLLIYAAS- T-LQSGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSYPPTFGPGTKVDIK
YU536-D04	DIQLTQSPSSLSASVGDRTITCRASQGISSYLA WYQQKPGKAPKLLIYAAS- T-LQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQLNSYPITFGQGTKLEIK
YU537-H11	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPG- TAPKLLIYDTNKRPSGIP- DRFSGSKSGTSATLGITGLQTGDEADYYCATWDSSLSVGVFGGGTKLTVL

Table 21.

mAb	Pairing abundance	pl human IgG	Germinality Index VH [%]	Germinality Index VL [%]	Unusual residues VH
STE90-C11	0.02	8	97.2	98	0
YU536-D04	0.02	8.39	95.4	96.9	0
YU537-H11	0.02	8.11	96.3	99	0

Table 22.

mAb	Unusual residues VL	Total CDR Length	Total hydrophobicity score	CDR Hydrophobicity score
STE90-C11	0	57	4.2	0.92
YU536-D04	0	57	4.37	1.45
YU537-H11	0	63	4.64	1.29

Table 23.

mAb	scFv charge	CDR charge total	Cysteines/Glycosylation Flags	Other PTM flags
STE90-C11	-1.04	-1.14	0	0
YU536-D04	1.22	-0.25	0	1
YU537-H11	0.82	-0.19	0	0

Table 24.

mAb	Amidation site	Asparagine deamidation site	Aspartate isomerization site	Cysteine in CDR
STE90-C11	0	1	1	0
YU536-D04	0	1	1	0
YU537-H11	0	0	3	0

Table 25.

mAb	Fragmentation site	Integrin binding site	Lysine glycation site	Methionine in CDR	N-glycosylation site
STE90-C11	0	0	0	1	0
YU536-D04	0	1	0	2	0
YU537-H11	0	0	0	2	0

Table 26.

mAb	N-terminal glutamate	Tryptophan in CDR
STE90-C11	0	0
YU536-D04	0	0
YU537-H11	1	1

Table 27.

mAb	ID - hIgG1 HS	Name	T cell Epitope Score	Critical Cys, N-Glyco
STE90-C11	TPP-27099	STE90-C11-hIgG1wt-HS-kappa	354.2	0
YU536-D04	TPP-27110	YU536-D04-hIgG1wt-HS-kappa	649.5	0
YU537-H11	TPP-27096	YU537-H11-hIgG1wt-HS-lambda	501.8	0

Table 28.

mAb	Non-Critical Asp Isomerisation, Deamidation, M-Oxidation (CDRs only M35 excluded)	Predicted Solubility [mg/ml]	Predicted thermostability [deg_C]	Predicted viscosity [cP]
STE90-C11	2	92.186	73.86	60.239
YU536-D04	3	89.391	76.308	26.142
YU537-H11	4	103.588	75.624	67.315

Table 29.

mAb			
STE90-C11	Kappa, G1	(IGKV1-9-01-IGKJ3-01)	(IGHV3-66-01-D-IGHJ3-01)
YU536-D04	Kappa, G1	(IGKV1-39-01-IGKJ2-01)	(IGHV3-66-01-D-IGHJ2-01)
YU537-H11	Lambda, G1	(IGLV1-51-01-IGLJ2-01)	(IGHV3-53-01-D-IGHJ1-01)

Example 11: Binding studies with “escape”-mutations

A deeper characterization of the best neutralizer was performed in respect to cross-reaction to other coronaviruses, binding specifically to known RBD mutations, biochemical and biophysical properties was conducted. The binding of STE90-C11, YU505-A02, YU537-H11 and YU536-D04 to other coronaviruses SARS-CoV-1, MERS-CoV, HCoV-HKU1, HCoV-229E and HCoV-NL63 Spike proteins was tested by titration ELISA. The antibody bound specifically SARS-CoV-2 and did not show any cross reactions to other human-pathogenic coronaviruses. The antibody CR3022 (Tian et al., 2020) was used as control, because of the known cross reactivity to SARS (data not shown).

Because first SARS-CoV-2 mutants in patients are being identified and mutations within the RBD have been characterized to arise under antibody selection pressure (Baum et al., 2020; Shi et al., 2020). S1 subunits harbouring single point mutations in the RBD were produced and binding of STE90-C11, YU505-A02, YU537-H11 and YU536-D04 to those mutants was tested.

A “worst-scenario” mutant containing all the 7 single point mutations, termed S1-7PM, was producible and therefore also tested. All constructs were still functionally binding recombinant ACE2.

The published antibodies anti-SARS-CoV-2 antibody CR3022 which is cross-reactive with SARS-CoV-2, the SARS-CoV-2 antibody CB6 (Shi et al., 2020) and the antibodies REGN10933 and REGN10987 (Hansen et al., 2020) were also titrated against the same panel of S1 mutants. The cumulative results of these experiments are summarized in Figure 30.

The antibodies STE90-C11, YU505-A02, YU537-H11 and YU536-D04 were still binding specifically to all of the investigated RBD mutations with only a slightly reduced binding specifically to the F486V mutation. Surprisingly, the antibodies still retain at least partially their binding activity to the “worst-case” S1-7PM mutant.

In contrast, CB6 showed reduced or strongly reduced binding specifically to four of the seven mutations. REGN10933 lost binding specifically to S1-7PM and showed reduced binding specifically to the F486V mutants. Binding to E484K, G485R seemed also to be at least reduced.

CR3022 showed also loss of binding specifically to the combined seven mutations and to the V367F mutation which is close to the antibody binding site and reduced binding specifically to the E484K and G485R mutation.

Example 12: Binning study

Epitope binning was performed in a premix setup. The antibodies were coupled in replicate (2X)

on a HC30M chip using EDC/NHS chemistry:

- 5µg/mL in acetate buffer pH 4.5, final volume 250µL
- Amount of antibody needed: 1.25µg
- Immobilization time 10min

Mixture of antibodies (1µM) and antigen (200nM) was prepared in running buffer (HBST), the final volume was 300µL.

- Amount of antibodies needed: 4µg
- Amount of antigen: RBD 40µg, S1-S2 177µg

Epitope binning setup: association 5 min, regeneration Glycine 1.5 2x40s.

Example 13: BLI-Measurement

In order to test the affinity of the inventive antibodies to the “escape”-mutants mentioned before, a BLI (bio-layer interferometry)-measurement was done (cf. also Abdiche et al., PLOS ONE, 1 March 2014 | Volume 9 | Issue 3).

BLI is a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time.

The binding between a ligand immobilized on the biosensor tip surface and an analyte in solution produces an increase in optical thickness at the biosensor tip, which results in a wavelength shift, $\Delta\lambda$, which is a direct measure of the change in thickness of the biological layer.

The affinity was measured by Bio-Layer Interferometry in three different assays using the Octet qKe (Fortebio/Sartorius GmbH, Göttingen, Germany).

In the first assay, anti-mouse Fc-Capture (AMC) sensors were activated for 10 min in PBS. After that, the sensors were equilibrated in assay buffer (PBS containing 1% BSA and 0.05% Tween 20) for 60 s before RBD-mFc (Sino Biologicals) was loaded onto the sensors at 10 µg/ml for 180 s. After a stable baseline measurement was established (60 s), antigen-loaded sensors were transferred to an 8-point antibody dilution series (500, 158, 50, 15, 5, 1.5, 0.5 and 0 nM). Association of the Fab antibody to the antigen was measured for 300 s. After that, the sensors were

transferred into assay buffer were the dissociation was measured for 900 s. Significant binding of the antibody to an unloaded sensor was not detected. For data analysis, the reference measurement (0 nM) was subtracted from the other measurements and data traces ranging from 158 to 1.5 nM were used for modelling of the kinetic data using a 1:1 binding model.

In the second assay, anti-human Fab (FAB2G) sensors were activated for 10 min in PBS. After that, the sensors were equilibrated in assay buffer (PBS containing 1% BSA and 0.05% Tween 20) for 60 s before the IgG antibody was loaded onto the sensors at 2.5 µg/ml for 180 s. After a stable baseline measurement was established (60 s), antibody-loaded sensors were transferred to an 8-point S1-HIS antigen dilution series (500, 158, 50, 15, 5, 1.5, 0.5 and 0 nM). Association of the S1 antigen to the antibody was measured for 300 s. After that, the sensors were transferred into assay buffer were the dissociation was measured for 900 s. Significant binding of the antigen to an unloaded sensor was not detected. For data analysis, the reference measurement (0 nM) was subtracted from the other measurements and data traces ranging from 50 to 5 nM were used for modelling of the kinetic data using a 1:1 binding model in the Data Analysis HT 11.0 software tool.

And in the third assay, Protein A sensors were activated for 10 min in PBS. Before use, the sensors were regenerated for 5 cycles in 10 mM Glycine buffer (pH2.0) followed by neutralization in PBS. Each step was performed for 5 s. After that, the regenerated sensors were equilibrated in assay buffer (PBS containing 1% BSA and 0.05% Tween 20) for 60 s before the IgG antibody was loaded onto the sensors at 2.5 µg/ml for 180 s. After a stable baseline measurement was established (60 s), antibody-loaded sensors were transferred to an 8-point S1-HIS antigen dilution series (500, 158, 50, 15, 5, 1.5, 0.5 and 0 nM). Association of the S1 antigen to the antibody was measured for 300 s. After that, the sensors were transferred into assay buffer were the dissociation was measured for 900 s. Significant binding of the antigen to an unloaded sensor was not detected. For data analysis, the reference measurement (0 nM) was subtracted from the other measurements and data traces ranging from 50 to 0.5 nM were used for modelling of the kinetic data using a 1:1 binding model.

Example 14: Affinity Study

STE90-C11, YU537-H11 and YU536-D04 affinities to different targets were measured by Bi-layer Interferometrie (BLI) (see example 13).

The binding-affinity of Fab antibodies to “wild-type” RBD-mFc (i.e. RBD without point mutations) immobilized on an anti-Fmc capture surface was as follows:

Antibody	K _D [nM]	R ²
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STE90-C11	8.14	0.9917
YU537-H11	61.1	0.9703
YU536-D04	17.5	0.9953

The binding-affinity to S1-His by immobilized Fab antibodies on a FAB2G- capture surface was as follows:

Antibody	K _D [nM]	R ²
STE90-C11	1.56	0.9969
YU537-H11	11.7	0.9963
YU536-D04	5.49	0.9917

The binding-affinity to S1-His by immobilized Fab antibodies on a Protein A-surface was as follows:

Antibody	K _D [nM]	R ²
STE90-C11	6.53	0.9891
YU537-H11	32.5	0.9798
YU536-D04	23	0.9964

Example 15: Binding study to RBD-mutations

The binding of STE90-C11 (SEQ ID. No.: 1305 – 1312) to S1 containing RBD mutations from COVID-19 patients was analyzed, showing a binding to most mutants. As the cell based inhibi-

tion analysis demonstrate, STE90-C11 is able to inhibit most analyzed mutants, validating a tolerance for RBD mutations. REGN10933 (Hansen et al., 2020), which is also binding at the RBD-ACE2 interface, showed a loss of neutralization in an assay using pseudoviral particles for the F486V and a reduced neutralization for both G485D and E484K mutations (Baum et al., 2020). Loss of binding to F486A is also described for VH-Fc ab8 (Li et al., 2020b). In the epitope analysis, REGN10933 has more molecular interactions within the region aa483-aa486 compared to STE90-C11. Currently, the variant B.1.1.7 is widely spread and STE90-C11 has reduced binding to N501Y and lost binding to mutants with the K417N/T mutations which occur in B.1.351 and B.1.1.28.1 variants.

Furthermore, STE90-C11 binds strongly to the RBD mutations in the emerging SARS-CoV-2 variants B.1.429/B.1.427 (L452R), B.1.526 (E484K or S477N), B1.258Δ (N439K), B.1.525 (E484K), B.1.1.28.2 (E484K) and B.1.1.33 (E484K). These variants are emerging, e.g. the frequency of B.1.429+B.1.427 reached 40% in January 2021 (Zhang et al., 2021) or B.1.258 with 59% in the samples sequenced in Czech in the last three month of 2020 (Brejová et al., 2021). B.1.617 (L452R, E484Q) has superseded B.1.1.7 in India and the prevalence of this variant was 100% of all sequenced viruses in mid of April 2021 (India Mutation Report. Alaa Abdel Latif, Julia L. Mullen, Manar Alkuzweny, Ginger Tsueng, Marco Cano, Emily Haag, Jerry Zhou, Mark Zeller, Nate Matteson, Chunlei Wu, Kristian G. Andersen, Andrew I. Su, Karthik Gangavarapu, Laura D. Hughes, and the Center for Viral Systems Biology. outbreak.info, (available at <https://outbreak.info/location-reports?loc=IND&selected=S%3AE484K&selected=B.1.1.7&selected=B.1.351&selected=B.1.617>). Accessed 21 April 2021).

Similar positive results were found for YU537-H11 (SEQ ID. No.: 389-408) and YU536-D04 (SEQ ID. No.: 349 – 368).

In summary, the antibodies YU537-H11 (SEQ ID. No.: 389-408), YU536-D04 (SEQ ID. No.: 349 – 368) and STE90-C11 (SEQ ID. No.: 1305 – 1312) bind to the RBD-ACE2 interface and maintain high similarity to the human germline V-genes VH3-66, the same family of many isolated anti-SARS-CoV-2 neutralizing antibodies disclosed herein. YU537-H11, YU536-D04 and STE90-C11 are tolerant to most known RBD mutants especially those of the mutants B.1.429/B.1.427, B.1.526, B1.258Δ, B.1.535, B.1.617 and B.1.1.33.

In a further binding study the antibody STE90-C11 (SEQ ID. No.: 1305 – 1312) was tested in its ability to bind to the “Indian mutant” (L452R+E484Q+D614G). It could be shown that STE-90-C11 bound this mutation as good as to the wildtype RBD (cf. figure 45).

Example 16: Development of conserved antibody sequences

From the findings above, a sequence alignment was performed with the software Clustal™

(Omega) in order to develop a concept of a conserved antibody sequence (cf. figures 43 & 44).

In a first alignment the heavy and light chain sequences of STE90-C11 and YU536-D04 were compared to each other (figure 43). A large number of identical, conserved and/or semi-conserved residues could be identified.

In another experiment an alignment of heavy and light chains of the antibodies STE90-C11, YU536-D04 with the further related antibody YU537-H11 was made (figure 44). Despite a more distant relationship of the YU537-cluster with the YU536- and STE90-cluster, a large number of identical, conserved and/or semi-conserved residues could be identified.

The alignments confirmed the earlier finding that the antibodies of the present invention represent a structurally related group of antibodies, which show advantageous features when it comes to the ability to recognize SARS-CoV-2-mutants.

Example 17: Animal studies

1. Hamster model of SARS-CoV-2 infection

Animal procedures were performed according to the European Guidelines for Animal Studies after approval by the Institutional Animal Care Committee and the relevant state authority (Landesamt für Gesundheit und Soziales, Berlin, Permit number 0086/20).

SARS-CoV-2 isolate BetaCoV/Germany/BavPat1/2020 (Wölfel et al., 2020) was used as challenge virus for hamster experiments. The virus was propagated and titrated on Vero E6 cells (ATCC CRL-1586) in minimal essential medium (MEM; PAN Biotech, Aidenbach, Germany) supplemented with 10% fetal bovine serum (PAN Biotech), 100 IU/ml penicillin G and 100 µg/ml streptomycin (Carl Roth, Karlsruhe, Germany) and stored at -80°C prior to experimental infections.

Per group, nine male and female Syrian hamsters (*Mesocricetus auratus*) strain RjHAN:AURA (Janvier, Le Genest-Saint-Isle, France) were used. Animals were housed in GR-900 IVC cages (Tecniplast, Buguggiate, Italy) and provided with food ad libitum and bountiful enrichment and nesting materials (Carfil, Oud-Turnhout, Belgium). Hamsters were randomly distributed into experimental groups and treated intraperitoneally with 3.7 mg/kg or 37 mg/kg STE90-C11 in a total volume of 1 ml PBS, two hours post infection, the control group received 1 ml PBS only at the same time-point.

SARS-CoV-2 infection was performed as previously described (Osterrieder et al., 2020). Briefly, anaesthetized hamsters received 1×10^5 pfu SARS-CoV-2 in 60 µL MEM intranasally two hours before treatment. Following infection, the clinical presentation of all animals was monitored

twice a day, body weight of all hamsters was recorded daily. On days 3, 5 and 14 post infection, three randomly assigned hamsters per group were euthanized. Euthanasia was applied by exsanguination under general anaesthesia as described (Nakamura et al., 2017). Oropharyngeal swabs and lungs were collected for virus titrations, RT-qPCR and/or histopathological examinations. All organs were immediately frozen at -80 °C or preserved in 4% formaldehyde for subsequent in-depth histopathological investigations.

To assess virus titers from 50 mg lung tissue, tissue homogenates were prepared using a bead mill (Analytic Jena) and 10-fold serial dilutions were prepared in MEM, and plated on Vero E6 cells in 12-well-plates. The dilutions were removed after 2 h and cells were overlaid with 1.25% microcrystalline cellulose (Avicel) in MEM supplemented with 10% FBS and penicillin/streptomycin. Three days later, cells were formalin-fixed, stained with crystal violet, and plaques were counted.

STE90-C11 was tested in a Syrian hamster challenge model. In this model, hamsters were intra-nasally infected with 1×10^5 pfu genuine SARS-CoV-2 and 2 h later treated with 3.7 mg/kg or 37 mg/kg STE90-C11 or with PBS in the control group. The virus titer in the lung of treated animals showed a dose dependent reduction of viral load on day 3 and 5 after infection. The measured mean viral load was 8.3×10^3 pfu with 37 mg/kg IgG compared to 1.5×10^6 pfu in the PBS control at day 3.

The hamster model is further characterized by a rapid weight loss in the first days after SARS-CoV-2 infection but also rapid recovery of body weight from around one week post infection on. Animals treated with the higher STE90-C11 dose showed a reduced loss of weight and a faster weight recovery compared to the PBS control.

2. Transgenic mice model of SARS-CoV-2 infection

All animal experiments were performed in compliance with the German Animal Welfare Act (TierSchG BGBl. I S. 1206, 1313; May 18, 2006) and Directive 2010/63/EU. The mice were handled in accordance with good animal practice as defined by the Federation for Laboratory Animal Science Associations and Gesellschaft für Versuchstierkunde/Society of Laboratory Animal Science. All animal experiments were approved by the responsible state office (Lower Saxony State Office of Consumer Protection and Food Safety) under permits number 20_3567.

K18hACE2 mice (B6.Cg-Tg (K18-ACE2)2PrImn/J) were purchased from Charles River (Sulzfeld, Germany), Mice were housed at the animal facility of the Helmholtz Centre for Infection Research under pathogen-free conditions.

Mice were fixed in a restrainer before injection and the lateral tail veins were hyperaemized. Different antibody concentrations were diluted in 100ul of PBS and injected intravenous into the

lateral tail vein.

Female and male at least 6-wk-old mice were infected with tissue culture– derived virus and housed in specific pathogen-free conditions throughout the experiment. Mice were anesthetized with Ketamin/Xylazin and inoculated intra-nasally with 2,000 PFU of virus in 20 μ l of Phosphate buffered saline (PBS).

The mice were sacrificed by CO₂ asphyxiation on day 5. Lungs were collected aseptically, homogenized in 500 μ L PBS and stored at -80°C . Part of the organ homogenates were used for titration cells and the other part for qPCR Analysis. Organ homogenates were serially diluted 1:10 – 1:105 in medium (DMEM supplemented with 5% FCS, 2 mM glutamine, 100 IU/mL penicillin and 100 μ g/mL streptomycin). Vero E-6 cells were then inoculated with 200 μ l of diluted homogenates and incubated for 1h, 37°C , CO₂ incubator. Cells were then covered with 1.75% Carboxymethyl-cellulose and incubated at 37°C , CO₂ incubator for 3-5 days. Plates were then fixed with 6% Paraformaldehyde for 1h and then stained with 1% Crystal violet. Plaques were then read under microscope.

The antibody STE90-C11 was tested in the transgenic human ACE2 mice model. Mice were first treated with 6, 30, 60 or 120 mg/kg STE90-C11 or with PBS in the control group. After 1h, they were inoculated intra-nasally with 1,000 or 2,000 pfu genuine SARS-CoV-2, or with PBS in the control group. The virus titer was measured with 4.7×10^4 pfu in the first experiment with 1,000 pfu and 7.4×10^5 pfu in the experiment with 2,000 pfu. Only in the two experiments, one with 60 mg/kg and one with 6 mg/kg a remaining viral load of 5.6×10^2 , respectively 3.1×10^2 pfu, was detected. In all other experiments the virus was completely removed from the lungs. In the control group, a significant weight loss was measured 4 days post infection but not the groups with antibody treatment.

Example 18: Selection pressure experiment

Calu-3 cells were seeded onto three 12-well plates. Antibody STE90-C11 or Palivizumab as IgG control was added in 5-fold dilutions (starting from 12 μ g/ml) to the growth medium. (12, 2.4, 0.48, 0.096, etc. μ g/ml). Then, SARS-CoV-2 was added (18.875 pfu/ml); CPE is monitored in the IncuCyte; CPE was visible at 3 dpi for COVEX-1 conc. 480 ng/ml and below and for Palivizumab in all concentrations.

Six days after infection, SN were taken from all antibody dilutions and those of 480 ng/ml COVEX-1 (IMP) and Palivizumab (12 μ g/ml) were transferred to naïve Calu-3 cells treated with the same conc. row of antibodies.

3.5 days after transfer, CPE was observed at conc. of 12 μ g/ml STE90-C11. SN from these

wells were inactivated following the Caliskan lab lysis protocol, transferred to cryotubes (2 replicates of each) and frozen at -80°C for further analysis.

Within the control typical mutations occurring randomly such as K417T and N501T with little or no selection advantage. Under selection pressure with STE90-C11 the mutation N501T (in the british mutant) was not increased (= blocked by STE90-C11), whereas the mutant K417T (in the brazilian mutant) was highly enriched und could not be repressed by STE90-C11.

STE90-C11 was also binding to the “Czech”, “Nigerian”, “Mink (Danish)” and “New York” mutations.

Example 19: Chain-shuffling experiment

In order to further show that the antibodies of the invention are structurally related with each other, a “chain-shuffling”-experiment was performed. That is, a hybrid-antibody comprising the heavy chain of YU536-D04 (Seq. ID No. 364) and the light chain of STE90-C11 (Seq. ID No. 1309) was produced and its binding ability to wildtype and mutant RBDs was measured (see result in figure 46).

In further experiments also other combinations were tested with similar results, such as depicted in table 30.

Table 30. Comparison of different hybrid antibodies

VL	VH	RBD-binding quality as compared to STE90-C11				wildtype virus neutralizing activity
		wt	N501Y	K417N	BSA	
STE90-C11 (SEQ ID No. 1309)	STE90-C11 (SEQ ID No. 1305)	+	-	-	-	+
YU536-D04 (SEQ ID No. 362)	STE90-C11 (SEQ ID No. 1305)	+	+	+	-	+
YU536-D04	YU537-H11	+	+	+	-	+

(SEQ ID No. 362)	(SEQ ID No. 404)					
STE90-C11 (SEQ ID No. 1309)	YU536-D04 (SEQ ID No. 364)	+	+	+	-	+
YU537-H11 (SEQ ID No. 402)	YU536-D04 (SEQ ID No. 364)	+	+	+	-	+
STE90-C11 (SEQ ID No. 1309)	YU537-H11 (SEQ ID No. 404)	+	+	+	-	+
YU537-H11 (SEQ ID No. 402)	STE90-C11 (SEQ ID No. 1305)	+	+	+	-	+
Control Antibody (Palivizumab)		-	-	-	-	-

Wherein “+” shows similar binding as compared to STE90-C11, and “-” shows little or no binding.

It turns out that also the hybrid-antibodies bind to the RBD with a very similar binding ability and show neutralizing activity. In case of mutants N501Y and K417N the binding of the hybrid-antibodies was even improved as compared to STE90-C11. This shows that the different heavy and light chains of the inventive antibodies can easily be combined with each other in order to archive a sufficient binding and even improve the binding to certain mutants, if needed.

Example 20: Comparison of neutralization activity of different antibodies

A comparison was done of some inventive antibodies with the prior art antibody COV2-2094 from Seth J. Zost et al.: „Potently neutralizing human antibodies that block SARS-CoV-2 receptor binding and protect animals”, bioRxiv, 22 May 2020. The results are depicted in table 31.

It turns out that the neutralizing activity of prior art antibody COV2-2094 is significantly less pronounced as the inventive antibodies.

Table 31. Results of neutralization comparison

Antibody	Neutralization
COV2-2094	1.03 nM
STE90-C11	0.56 nM
YU536-D04	0.40 nM
YU537-H11	0.47 nM

Claims

1. A humanized or a human therapeutic monoclonal antibody or antigen-binding fragment, or a substantially similar variant thereof that binds specifically to SARS-CoV-2 glycoprotein S with the amino acid sequence of SEQ ID NO: 02 and neutralizes the infectious activity of SARS-CoV-2.
2. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to claim 1, wherein the neutralization of the infectious activity of SARS-CoV-2 results in confluence score of at least 95% as measured with an infectious SARS-CoV-2 VERO E6 neutralizing assay.
3. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any of claims 1 or 2, wherein the antibody or antigen-binding fragment binds specifically to an epitope within the receptor binding domain (RBD) of the S1 domain of SARS-CoV-2 glycoprotein (SEQ ID NO: 06) and binds specifically to at least one of the escape-mutations of the S1 domain of SARS-CoV-2 glycoprotein selected from the list consisting of S1-V367F, S1-N439K, S1-G476S, S1-V483A, S1-E484K, S1-G485R, S1-F486V, S1-7PM-mutant (SEQ ID NO: 1730), and any combination thereof.
4. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to claim 3, wherein said antibody or antigen-binding fragment thereof binds specifically both to an epitope comprising the amino acids 400-FVIR-GDEV RQIAPQTGKIADDYN-422 within the RBD (SEQ ID NO: 06) as well as to the S1-7PM-mutant (SEQ ID NO: 1730).
5. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any of claims 1 or 2, wherein said antibody or antigen-binding fragment thereof competes for binding to an epitope within the RBD (SEQ ID NO: 06) with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305; and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309 or with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928; and wherein the contamination ratio is less than 0.2.
6. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any of claims 1 or 2, wherein said antibody or antigen-binding fragment thereof competes for binding to the epitope 165-AKRVNCYFPLQSYGFQ-180 within the S1-7PM-mutant (SEQ ID NO: 1730) with an antibody comprising a heavy

chain variable region of the amino acid sequence in SEQ ID NO: 1305; and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309 or with an antibody comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928.

7. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any of the previous claims, comprising

a heavy chain CDR1 (H-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1306, 910, 350, and 390;

a heavy chain CDR2 (H-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1307, 912, 352, and 392;

a heavy chain CDR3 (H-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1308, 914, 354, and 394;

and

a light chain CDR1 (L-CDR1) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1310, 916, 356, and 396;

a light chain CDR2 (L-CDR2) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1311, 918, 358, and 398;

a light chain CDR3 (L-CDR3) domain with an amino acid sequence selected from the group consisting of SEQ ID NO: 1312, 920, 360, and 400.

8. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any one of the previous claims, selected from the group of antibodies comprising a heavy chain variable region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 926 and a light chain variable region of the amino acid sequence in SEQ ID NO: 928; a heavy chain variable region of the amino acid sequence in SEQ ID NO: 366 and a light chain variable region of the amino acid sequence in SEQ ID NO: 368; and/or a heavy chain variable region of the amino acid sequence in SEQ ID NO: 406 and a light chain variable region of the amino acid sequence in SEQ ID NO: 408; and/or any combinations thereof.
9. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any one of the previous claims, comprising a heavy chain variable

region of the amino acid sequence in SEQ ID NO: 1305 and a light chain variable region of the amino acid sequence in SEQ ID NO: 1309.

10. The therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any of the previous claims, characterized by one or more of the following:
 - capable of binding specifically to the RBD of SARS-CoV-2 Glycoprotein S with an affinity (K_D) of 6.5×10^{-8} M or less, preferably 10×10^{-9} M or less,
 - capable of reducing or preventing an antibody-dependent enhancement (ADE) reaction in a patient,
 - capable of preventing, reducing or healing a viral infection with SARS-CoV-2,
 - capable of reducing or preventing the cytokine storm syndrome (CSS) in a patient,
 - capable of reducing the load of viral particles in the sputum of a patient by at least 50% for at least 48 hours after administration of the therapeutic antibody or antigen-binding fragment thereof,
 - having a specific binding specifically to the S1-7PM-mutant (SEQ ID: 1730) with a K_D of 10×10^{-9} M or less,
 - having an IC_{50} of less than 0.036 $\mu\text{g/ml}$, preferably of 0.026 $\mu\text{g/ml}$ or less.
11. An isolated nucleic acid molecule encoding the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any one of the previous claims.
12. An expression vector comprising the nucleic acid molecule according to claim 11.
13. A method of producing the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any one of the previous claims comprising the steps of
 - introducing the expression vector according to claim 12 into an isolated host cell,
 - growing the cell under conditions permitting production of the antibody or antigen-binding fragment thereof ,
 - recovering the antibody or fragment so produced.

14. A pharmaceutical composition comprising the therapeutic antibody or antigen-binding fragment or a substantially similar variant thereof according to any one of claims 1 to 10, and a pharmaceutically acceptable carrier.
15. A therapeutic antibody or antigen-binding fragment according to any one of claims 1 to 10 for the use in the prevention and/or treatment of a SARS-CoV-2-related disease or condition in a patient.