

**Table S1: CV9103 antigens PSA, PSMA, PSCA and STEAP1**

| <b>Antigen</b> | <b>Full name</b>                                                                              | <b>Function</b>             | <b>Localization</b>                     |
|----------------|-----------------------------------------------------------------------------------------------|-----------------------------|-----------------------------------------|
| PSA            | <u>P</u> rostate- <u>s</u> pecific <u>a</u> ntigen (KLK3, kallikrein-related peptidase 3)     | Serine protease             | Extracellular                           |
| PSCA           | <u>P</u> rostate <u>s</u> tem <u>c</u> ell <u>a</u> ntigen                                    | Unknown                     | Cell surface, anchored by a GPI linkage |
| PSMA           | <u>P</u> rostate- <u>s</u> pecific <u>m</u> embrane <u>a</u> ntigen (FOLH1, folate hydrolase) | Glutamate carboxypeptidase  | Transmembrane glycoprotein (Type II)    |
| STEAP1         | <u>S</u> ix- <u>t</u> ransmembrane <u>e</u> pithelial <u>a</u> ntigen of the prostate 1       | Intercellular communication | Transmembrane glycoprotein (Type I)     |

GPI, glycosylphosphatidylinositol

**Table S2: Eligibility criteria**

| Inclusion criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Exclusion criteria                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
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| <ul style="list-style-type: none"> <li>• Signed informed consent in accordance with GCP and local regulatory requirements prior to trial participation</li> <li>• Male and age <math>\geq 18</math> years</li> <li>• Histologically confirmed diagnosis of adenocarcinoma of the prostate, Gleason score available</li> <li>• Patients must have been treated with hormonal therapy and may have been treated with surgery and/or radiation therapy (see exclusion criteria)</li> <li>• Progressive disease as defined by hormone refractoriness and rise in PSA. Hormone refractoriness: defined by rise in PSA, and/or RECIST-based progression of evaluable lesions, and/or increased number of hotspots on a bone scan, while the patient has a castrated level of testosterone. This castrated level may have been obtained by orchiectomy or LH-RH analog <math>\pm</math> AA. AA must be discontinued for <math>\geq 4</math> weeks before study entry to exclude a withdrawal effect. Rise in PSA: rise in PSA level on 3 consecutive time points (PSA rise over nadir, separated by <math>&gt;1</math> week, PCWG2 criteria)</li> <li>• Presence of metastatic disease is acceptable</li> <li>• ECOG performance status of 0 to 1</li> <li>• Life expectancy <math>&gt;12</math> months</li> <li>• Adequate organ function</li> <li>• Bone marrow function: hemoglobin <math>\geq 10</math> mg/dL; leukocytes <math>\geq 3,000/\mu\text{L}</math>; lymphocytes <math>\geq 1,000/\mu\text{L}</math>; absolute neutrophil count <math>\geq 1,500/\mu\text{L}</math>; platelet count</li> </ul> | <ul style="list-style-type: none"> <li>• Other histologic type of prostate cancer (transitional cell, small cell, or squamous cell cancer)</li> <li>• Symptomatic brain metastasis or leptomeningeal involvement</li> <li>• Patient receiving systemic anticancer therapy</li> <li>• Symptomatic congestive heart failure (NYHA 3 and 4); unstable angina pectoris; significant cardiac arrhythmia</li> <li>• Pulmonary disease causing dyspnea or fatigue during normal activity; active significant infection</li> <li>• History of seizures, encephalitis, or multiple sclerosis</li> <li>• Documented history of active autoimmune disorders requiring systemic immunosuppressive therapy (e.g. sarcoidosis, lupus erythematosus, rheumatoid arthritis, glomerulonephritis, or systemic vasculitis), excepting autoimmune thyroiditis with only thyroid hormone replacement and stable disease <math>&gt;1</math> year inflammatory bowel disease e.g. Crohn's disease or ulcerative colitis; active diverticulitis</li> <li>• Primary or secondary immune deficiency</li> <li>• History of allergy requiring medication</li> <li>• Active drug abuse or chronic alcoholism</li> <li>• Clinically significant active infections</li> <li>• Seropositive for HIV, HBV, or HCV</li> </ul> |

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| <p>≥100,000/μL</p> <ul style="list-style-type: none"> <li>• Renal: creatinine ≤1.5 mg/dL or creatinine clearance ≥60 mL/min; hepatic: AST and ALT ≤2.5 times ULN; bilirubin ≤1.5 ULN; HBV and HCV negative</li> <li>• Concomitant LH-RH therapy continuation is acceptable</li> <li>• May have had local palliative radiotherapy for bone metastasis involving &lt;25% of bone marrow</li> <li>• Patients requiring bisphosphonates at the time of registration into the trial are eligible (therapy initiated ≥28 days prior to first study treatment administration) and must be continued at a constant level during the study period</li> <li>• Patients of child-producing potential must agree to use contraception while enrolled in the study and for 1 month after the last immunization</li> </ul> | <ul style="list-style-type: none"> <li>• History of other malignancies over the last 5 years (except basal cell carcinoma of the skin or carcinoma <i>in situ</i> of the bladder)</li> <li>• Uncontrolled medical condition considered as high risk for treatment with an investigational drug, including unstable diabetes mellitus, vena-cava-syndrome, known ascites, and/or pleural effusion, symptomatic pleural effusion treated by puncture</li> <li>• Renal insufficiency requiring dialysis</li> <li>• Patients who are planning to father a child/not willing to use a barrier method of contraception</li> </ul> |
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AA, anti-androgen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECOG, Eastern Cooperative Oncology Group; GCP, good clinical practice; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; LH-RH, luteinizing hormone-releasing hormone; NYHA, New York Heart Association; PCWG, Prostate Cancer Working Group; PSA, prostate-specific antigen; RECIST, Response Evaluation Criteria in Solid Tumors; ULN, upper limit of normal

**Table S3: Selected epitopes of CV9103 antigens**

|                                    | <b>PSA</b>                                           | <b>PSCA</b>                                           | <b>PSMA</b>                            | <b>STEAP1</b>                         |
|------------------------------------|------------------------------------------------------|-------------------------------------------------------|----------------------------------------|---------------------------------------|
| <b>A1</b>                          | VSHSFPHPLY<br>PSLYTKVVHY<br>ALPERPSLY                | LQPGTALLCY<br>CVDDSDQDYY                              | HSTNGVTRIIY<br>ETYELVEKFY<br>AGDPLTPGY | EHDVWRMEIY<br>WIDIKQFVWY<br>ATSHQQYFY |
| <b>A2</b>                          | FLTPKKLQCV<br>KLQCVDLHV<br>VISNDVCAQV                | ALQPGTALL<br>ALLPALGLL                                | LLHETDSAV<br>ALFDIESKV<br>VLAGGFLL     | LLLGTIHAL<br>FLYTLLREV<br>LLLGTIHAL   |
| <b>A3</b>                          | GAAPLILSR<br>QVHPQKVTK<br>AVCGGVLVH                  | AVGLLTVISK<br>RIRAVGLLT<br>ALLMAGLAL                  | KVFRGNKVK<br>STEWAEENSR<br>RLLQERGVAY  | IVQLHNGTK<br>SITLLALVY<br>AIVQLHNGTK  |
| <b>A24</b>                         | CYASGWGSI<br>HYRKWIKDTI                              | DYYVGKKNI                                             | NYARTEDFF<br>LYSDPADYF                 | VFLPIVLI<br>QYFYKIPIL                 |
| <b>B7</b>                          | TPKKLQCVDL<br>RPGDDSSHDL<br>GPLVCNGVL                | QPAAAILAL                                             | RPRWLCAGAL<br>LPSIPVHPI<br>RPRRTILFA   | IVQLHNGTK<br>ILFLPCLRK<br>AILALLAVTS  |
| <b>B8</b>                          | CIRNKSUIL<br>LLKNRFLRP<br>MSLLKNRFL                  | TARIRAVGL<br>TVISKGCSL                                | GNKVKNAQL<br>GWRPRRTIL<br>LAGAKGVIL    | CLRKKILKI<br>MLTRKQFGL                |
| <b>B35</b>                         | EPALGTTCY<br>HPEDTGQVF                               | QPGTALLCY<br>QPAAAILAL                                | DPLGLPDRPF<br>FPGIYDALF                | HPLATSHQQY<br>TPPTFMIAVF              |
| <b>B44</b>                         | EEFLTPKKL<br>QEPALGTTCY                              | GEQCWTARI<br>ARIRAVGLL                                | NEIFNTSLF<br>KEFGLDSVEL<br>HEIVRSFGTL  | DEFDCPSEL<br>SELQHTQEL<br>REFHYIQSKL  |
| <b>DRB1*01</b>                     | TLSVTWIGAAPLILS<br>ASGWGSIEPEEFLTP                   | AAILALLPALGLLL<br>AVLLALLMAGLALQP                     | FLLGLFLGWFIKSS<br>QKLEKMGGSAPPDS       | SFFFAVLHAIYSLSY<br>LLALVYLPGVIAAIV    |
| <b>DR B1*03</b>                    | CGGVLVHPQWVLTAA<br>NKSVILLGRHSLFHP                   | SLNCVDDSDQDYYVGK<br>ILALLPALGLLLWGP                   | ISIINEDGNEIFNTS<br>GVILYSDPADYFAPG     | DKWMLTRKQFGLLSF<br>SYPMRRSYRYKLLNW    |
| <b>DR B1*04</b>                    | HSLFHPEDTGQVFQV<br>QVFQVSHSFPHPLYD                   | AVLLALLMAGLALQP<br>KAQVSNEDCLQVENC                    | STEWAEENSRLQER<br>VKNFTEIASKFSERL      | LTFLYTLLREVIHPL<br>PGVIAAIVQLHNGTK    |
| <b>DR B1*07</b>                    | VPVVFLTLSVTWIGA<br>HPQWVLTAAHCIRNK                   | LNCVDDSDQDYYVGKK<br>ILALLPALGLLLWGP                   | GMVFELANSIVLPFD<br>MKTYSVSFDSLFSAV     | IAAIIASLTFLYTLL<br>IHALIFAWNKWIDIK    |
| <b>DR B1*011</b>                   | PSLYTKVVHYRKWIK<br>TGQVFQVSHSFPHPPL                  | VGLLTVISKGCSLNC<br>EQCWTARIRAVGLLT                    | LYNFTQIPHLAGTEQ<br>CRDYAVVLRKYADKI     | KIPILVINKVLPMSV<br>YTLLREVIHPLATSH    |
| <b>DR B1*015</b>                   | LQGITSWGSEPCALP<br>SRIVGGWECEKHSQP<br>KKLQCVDLHVVISN | LALLMAGLALQPGTA<br>AHALQPAAAILALLP                    | SDIVPPFSAFSPQGM<br>HEIVRSFGTLKKEGW     | VWRMEIYVSLGIVGL<br>TFMIAVFLPIVVLIF    |
| <b>Promiscuitiv<br/>e epitopes</b> | WQVLVASRGRAVCGG<br>SVTWIGAAPLILSRI                   | TARIRAVGLLTVISK<br>AHALQPAAAILALLP<br>ILALLPALGLLLWGP | VKNFTEIASKFSERL<br>NYTLRVDCTPLMYSL     | LSFFFAVLHAIYSLS<br>INKVLPMVSITLLAL    |

SA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; STEAP1, 6-transmembrane epithelial antigen of the prostate 1

**Table S4: Summary of all immunologically evaluable patients.** Response against antigen, type of detected response, time of detected response and fold increase above baseline are summarized.

| Pt | Dose | antibody |             | CD4 |         |             |          | CD8 |         |             |          |
|----|------|----------|-------------|-----|---------|-------------|----------|-----|---------|-------------|----------|
|    |      | ±        | Week of max | ±   | Antigen | Week of max | Max fold | ±   | Antigen | Week of max | Max fold |
| 1  | I    | neg      |             | neg |         |             |          | pos | PSA     | 17          | 4.33     |
|    |      |          |             |     |         |             |          | pos | PSMA    | 9           | 3        |
|    |      |          |             |     |         |             |          | pos | STEAP1  | 17          | 5        |
| 2  | I    | neg      |             | neg |         |             |          | neg |         |             |          |
| 3  | I    | neg      |             | neg |         |             |          | neg |         |             |          |
| 4  | II   | neg      |             | neg |         |             |          | neg |         |             |          |
| 5  | II   | pos      | 17          | neg |         |             |          | neg |         |             |          |
| 6  | III  | neg      |             | neg |         |             |          | pos | PSA     | 17          | 8        |
| 7  | III  | neg      |             | pos | PSA     | 9           | 2.56     | neg |         |             |          |
| 8  | III  | pos      | 9           | neg |         |             |          | neg |         |             |          |
| 9  | III  | neg      |             | neg |         |             |          | neg |         |             |          |
| 10 | III  | pos      | 17          | pos | PSMA    | 9           | 2        | neg |         |             |          |
| 11 | III  | neg      |             | pos | PSMA    | 17          | 7        | neg |         |             |          |
|    |      |          |             |     | STEAP1  | 17          | 8.25     |     |         |             |          |
| 12 | III  | neg      |             | neg | STEAP1  | 17          | 2        | neg |         |             |          |
| 13 | III  | neg      |             | neg |         |             |          | neg |         |             |          |
| 14 | III  | neg      |             | pos | PSA     | 9           | 8        | neg |         |             |          |
|    |      |          |             |     | PSCA    | 9           | 2        |     |         |             |          |
|    |      |          |             |     | STEAP1  | 17          | 3        |     |         |             |          |
| 15 | III  | neg      |             | neg |         |             |          | neg |         |             |          |
| 16 | III  | neg      |             | pos | PSCA    | 5           | 2        | neg |         |             |          |
| 17 | III  | neg      |             | pos | PSA     | 17          | 2        | neg |         |             |          |
|    |      |          |             |     | PSCA    | 9           | 17.6     |     |         |             |          |
|    |      |          |             |     | PSMA    | 17          | 25       |     |         |             |          |
|    |      |          |             |     | STEAP1  | 17          | 34.5     |     |         |             |          |
| 18 | III  | neg      |             | neg |         |             |          | pos | PSMA    | 17          | 3.33     |
|    |      |          |             |     |         |             |          |     | STEAP1  | 17          | 5.6      |
| 19 | III  | neg      |             | neg |         |             |          | neg |         |             |          |
| 20 | III  | neg      |             | pos | PSMA    | 9           | 2.2      | pos | PSA     | 9           | 3.5      |
| 21 | III  | neg      |             | neg |         |             |          | pos | PSA     | 9           | 2.39     |
|    |      |          |             |     |         |             |          |     | PSMA    | 17          | 3.83     |
| 22 | III  | neg      |             | neg |         |             |          | neg |         |             |          |
| 23 | III  | neg      |             | pos | PSMA    | 17          | 44       | pos | PSMA    | 17          | 15.63    |
|    |      |          |             |     | STEAP1  | 17          | 21       |     | STEAP1  | 17          | 11.13    |

|    |     |     |    |     |        |    |      |     |        |    |      |
|----|-----|-----|----|-----|--------|----|------|-----|--------|----|------|
| 24 | III | neg |    | neg |        |    |      | neg |        |    |      |
| 25 | III | neg |    | neg |        |    |      | pos | PSA    | 9  | 2.75 |
|    |     |     |    |     |        |    |      |     | PSCA   | 5  | 4    |
| 26 | III | neg |    | pos | PSMA   | 17 | 3    | neg |        |    |      |
| 27 | III | neg |    | neg |        |    |      | pos | PSA    | 17 | 126  |
|    |     |     |    |     |        |    |      |     | PSMA   | 9  | 3    |
|    |     |     |    |     |        |    |      |     | STEAP1 | 17 | 2    |
| 28 | III | neg |    | pos | PSA    | 17 | 8    | pos | PSA    | 17 | 8    |
|    |     |     |    | pos | PSCA   | 9  | 7    |     | PSCA   | 9  | 3    |
|    |     |     |    | pos | STEAP1 | 9  | 4    |     | PSMA   | 9  | 4    |
|    |     |     |    | neg |        |    |      |     | STEAP1 | 9  | 22.6 |
| 29 | III | pos | 17 | neg |        |    |      | pos | PSA    | 5  | 3.88 |
| 30 | III | neg |    | pos | PSA    | 9  | 3.05 | pos | PSCA   | 9  | 2    |
|    |     |     |    | pos | PSMA   | 9  | 15.4 |     |        |    |      |
| 31 | III | neg |    | pos | PSMA   | 9  | 8.45 | pos | PSA    | 5  | 7    |
| 32 | III | neg |    | neg |        |    |      | pos | PSCA   | 17 | 2    |
|    |     |     |    |     |        |    |      |     | PSMA   | 17 | 2    |
|    |     |     |    |     |        |    |      |     | STEAP1 | 17 | 2.25 |
| 33 | III | pos |    | neg |        |    |      | pos | PSCA   | 9  | 2.53 |
|    |     |     |    |     |        |    |      |     | PSMA   | 9  | 2.5  |
| 34 | III | neg |    | pos | PSA    | 9  | 9    | neg |        |    |      |
| 35 | III | neg |    | neg |        |    |      | pos | PSA    | 9  | 14.5 |
| 36 | III | neg |    | neg |        |    |      | neg |        |    |      |
| 37 | III | neg |    | neg |        |    |      | pos | STEAP1 | 9  | 4    |
| 38 | III | neg |    | neg |        |    |      | pos | PSMA   | 17 | 9    |

CD, cluster of differentiation; max, maximum; mRNA, messenger ribonucleic acid; PSA, prostate-specific antigen; PSCA, prostate stem cell antigen; PSMA, prostate-specific membrane antigen; STEAP1, 6-transmembrane epithelial antigen of the prostate 1

**Table S5: Number of subjects receiving 1, 2, 3, 4, or 5 vaccinations per cohort**

| <b>Dose level/number of subjects</b> | <b>I (N=3)</b> | <b>II (N=3)</b> | <b>III (N=38)</b> | <b>All (N=44)</b> |
|--------------------------------------|----------------|-----------------|-------------------|-------------------|
| 1 vaccination                        |                |                 |                   |                   |
| 2 vaccinations                       |                | 1               | 1                 | 2                 |
| 3 vaccinations                       |                |                 | 2                 | 2                 |
| 4 vaccinations                       | 2              |                 | 7                 | 9                 |
| 5 vaccinations                       | 1              | 2               | 28                | 31                |

**Table S6: Antigen-specific responses for patients responding to only one antigen.**

|            | PSA | PSCA | PSMA | STEAP |
|------------|-----|------|------|-------|
| Patient 1  | +   |      |      |       |
| Patient 2  | +   |      |      |       |
| Patient 3  | +   |      |      |       |
| Patient 4  |     |      |      | +     |
| Patient 5  |     | +    |      |       |
| Patient 6  |     |      | +    |       |
| Patient 7  | +   |      |      |       |
| Patient 8  | +   |      |      |       |
| Patient 9  | +   |      |      |       |
| Patient 10 |     |      |      | +     |
| Patient 11 |     |      | +    |       |

**Table S7: Antigen-specific responses for patients responding to two or more antigens.**

|            | <b>PSA</b> | <b>PSCA</b> | <b>PSMA</b> | <b>STEAP</b> |
|------------|------------|-------------|-------------|--------------|
| Patient 1  | +          |             | +           |              |
| Patient 2  |            |             | +           | +            |
| Patient 3  | +          | +           |             | +            |
| Patient 4  | +          | +           | +           | +            |
| Patient 5  |            |             | +           | +            |
| Patient 6  | +          |             | +           |              |
| Patient 7  | +          |             | +           |              |
| Patient 8  |            |             | +           | +            |
| Patient 9  | +          | +           |             |              |
| Patient 10 | +          |             | +           | +            |
| Patient 11 | +          | +           | +           | +            |
| Patient 12 | +          | +           | +           |              |
| Patient 13 | +          |             | +           |              |
| Patient 14 |            | +           | +           | +            |
| Patient 15 | +          | +           | +           |              |