# Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival

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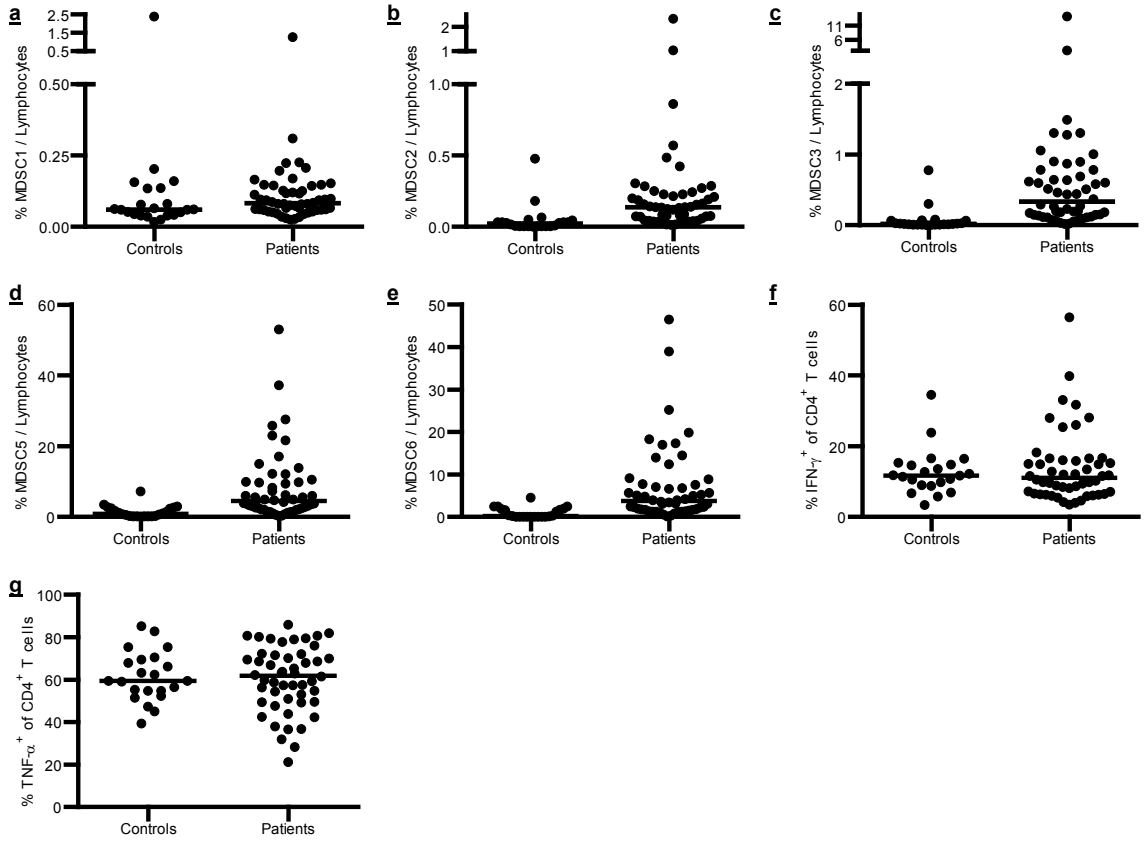
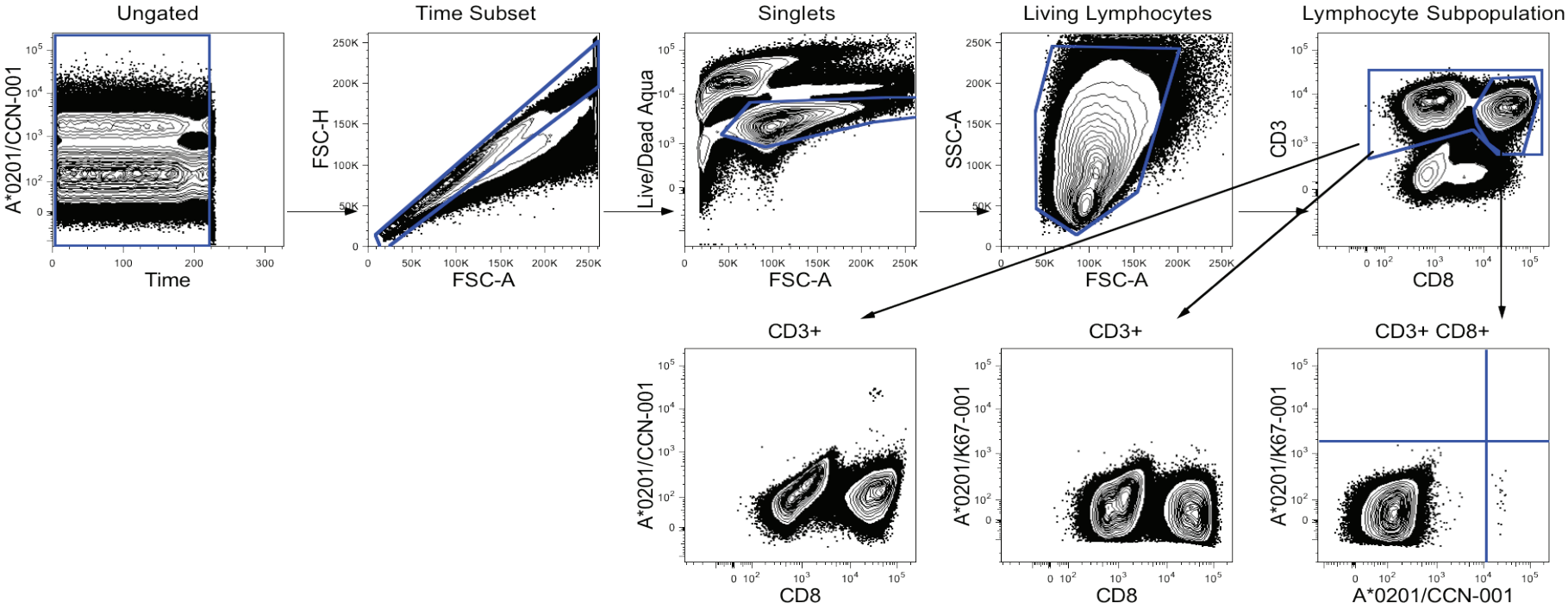



**Supplementary Figure 1: Analysis of pre-treatment biomarkers.**
Comparison of pre-treatment cellular biomarkers of evaluable ITT patients aged < 70 years (N=50-52) and age/gender-matched healthy controls (N=22). (●) represent individuals and (—) represent medians. (a) MDSC1 p=0.091, (b) MDSC2 p<0.0001, (c) MDSC3 p<0.0001, (d) MDSC5 p<0.0001, (e) MDSC6 p<0.0001, (f) IFN-$\gamma^+$ p=0.73 and (g) TNF-$\alpha^+$ $T_H$ subset p=0.99.

Walter et al., Supplementary Figure 2



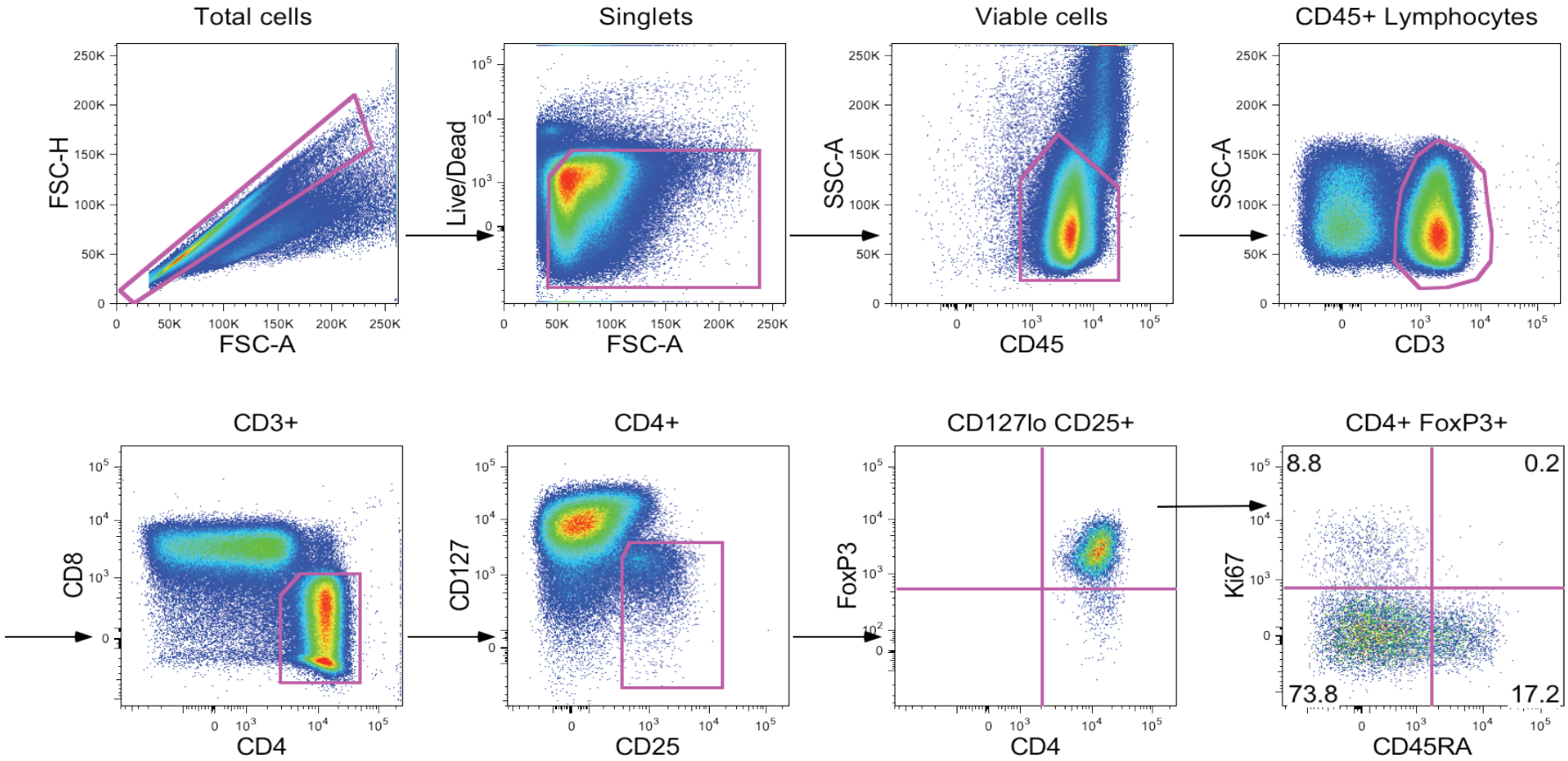

**Supplementary Figure 2: Flow cytometry analysis of one representative vaccine-induced response of the IMA901 phase II study with detailed gating strategy.**

Staining was performed using a live/dead marker, CD3- and CD8-antibodies and two different PE- and APC-labeled multimeric HLA/peptide complexes. Due to the high variability of the HLA multimer staining to individual patient T-cell populations, a uniform gating for the entire study was not feasible, however all gates were kept identical for each patient and antigen. First, singlets were identified by setting a time gate followed by exclusion of doublets using an FSC-A vs. FSC-H plot. Within the singlets living lymphocytes were identified by gating dead cell marker negative cells and subsequently a lymphocyte subpopulation was defined by gating FSC-A vs. SSC-A. Next, gates were set around $CD3^+$ and $CD3^+CD8^+$ cells respectively. Stainings for PE-labeled or APC-labeled multimers were then visualized first within the $CD3^+$ subset by plotting each multimer staining vs. CD8 and finally in a multimer vs. multimer plot showing $CD3^+CD8^+$ cells to identify single multimer binding T cells.

Walter et al., Supplementary Figure 3


**Supplementary Figure 3**

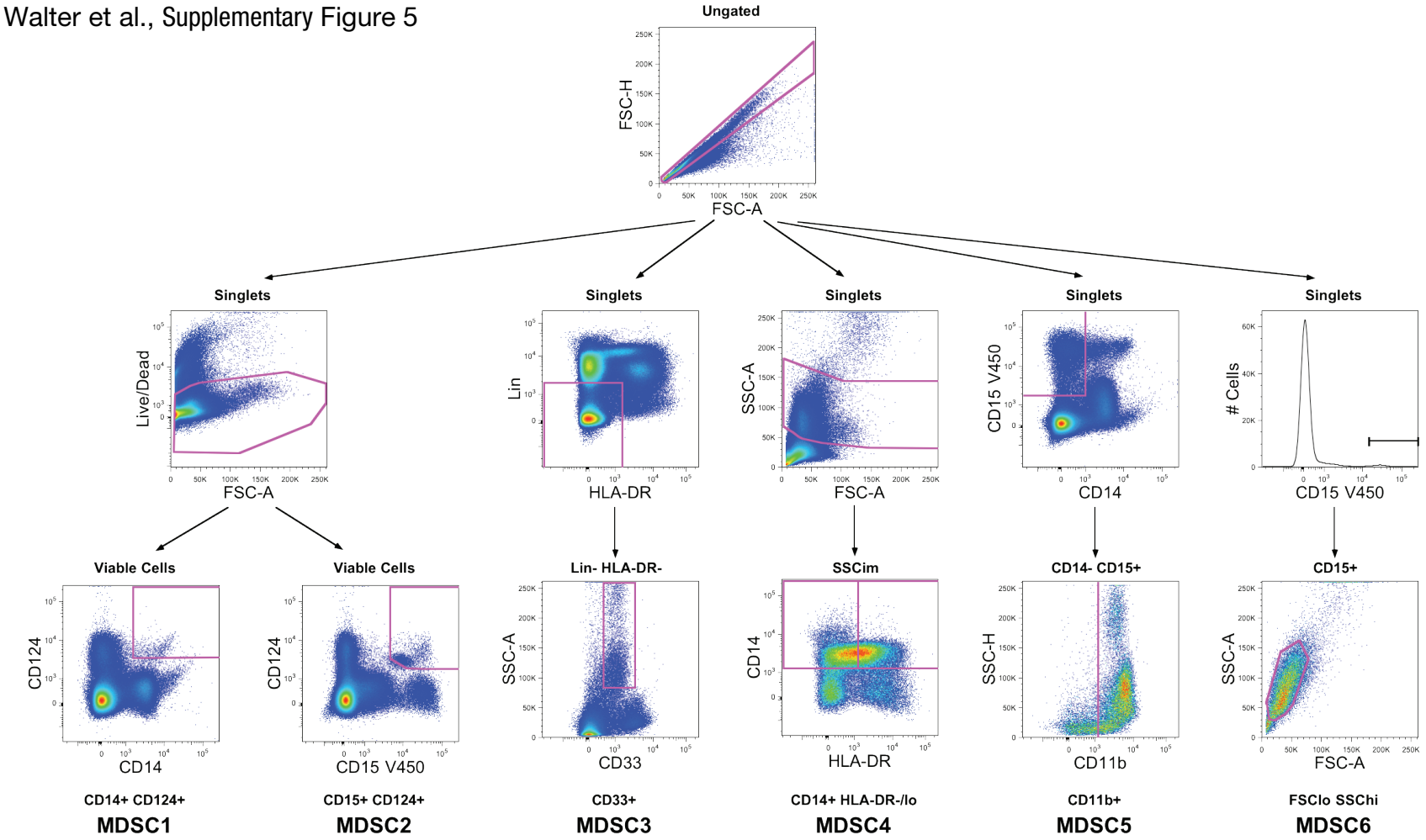
**Flow cytometry plots of 20 vaccine-induced responses of the IMA901 phase II study.** Staining and gating analysis was performed as detailed in legend to Supplementary Figure 2. For each patient two plots of the pre-vaccination time-point and a post-vaccination time-point are shown: (1.) CD8 vs. multimer-1 showing $CD3^+$ cells and (2.) multimer-1 vs. multimer-2 showing $CD3^+CD8^+$ cells, the latter allowing demonstration of peptide specific multimer binding. A response was determined as vaccine-induced if the frequency of multimer-positive cells in the respective post-vaccination time-point was at least 4x the frequency compared to the pre-vaccination time-point, and the population of multimer-positive cells was classified as clustered and discrete by a jury consisting of five members. Jury members individually inspected each plot which was blinded for patient data and multimer specificity. Each multimer plot received a score of 0 (clearly negative), 1 (possibly positive) or 2 (clearly positive) by each jury member. Each staining could therefore receive a maximum of 2x5 = 10 points. A multimer staining was considered as clustered and discrete if its total score was at least 9. Vaccine-induced responses from 10 patients treated with and from 10 patients treated without low-dose cyclophosphamide are shown.




**Supplementary Figure 4**

**Representative flow cytometry plots showing the detailed gating strategy for quantification of regulatory T cells (Tregs).** Identical gates were used for analyses of all study samples. Samples were set as evaluable if at least 75,000 live CD45$^+$ lymphocytes were counted. To quantify Tregs, first total cells were identified by excluding debris using an FSC-A histogram. Singlets were selected as defined by having a similar height (FSC-H) and area (FSC-A) measurement in the forward scatter. Next, a gate was set to identify viable cells. Then CD45$^+$ lymphocytes and CD3$^+$ cells were selected. The CD4$^+$ CD8$^-$ subset among CD3$^+$ cells was identified and plotted for CD25 and CD127 expression. FoxP3$^+$ cells within CD127$^{lo}$ CD25$^+$ cells were identified as Tregs. Last, Tregs were characterized in terms of Ki67 and CD45RA expression which allows quantification of Treg subpopulations such as dividing vs. non-dividing (Ki67$^+$ vs. Ki67$^-$).

Walter et al., Supplementary Figure 5




**Supplementary Figure 5**

**Representative flow cytometry plots showing the detailed gating strategy for quantification of myeloid-derived suppressor cells (MDSCs).** Identical gates were used for analyses of all study samples. Samples were set as evaluable if at least 75,000 live CD45$^+$ lymphocytes were counted. To quantify MDSCs, doublets corresponding to cell aggregates were excluded by the FSC-A/FSC-H profile and single cells were further analyzed. Progressive gating was next performed on singlet cells for all MDSC populations. For MDSC populations 1 and 2 a live/dead gating was included to avoid non-specific binding of anti-CD124 to dead cells.

MDSC1: gated on viable cells and identified as CD14$^+$ CD124$^+$

MDSC2: gated on viable cells and identified as CD15$^+$ CD124$^+$

MDSC3: gated on Lin$^-$ HLA-DR$^-$ CD33$^+$ (whereby Lin is CD3/14/19/56)

MDSC4: gated on CD14$^+$ HLA-DR$^{-/lo}$

MDSC5: gated on CD14$^-$ CD15$^+$ CD11b$^+$

MDSC6: gated CD15$^+$ FSC$^{lo}$ SSC$^{hi}$

**Supplementary Table 1: Baseline characteristics and survival of IMA901-202 patients (PP).**

| | | All | +CY | –CY |
|---|---|---|---|---|
| **Sample size (*n*)** | | 64 | 31 | 33 |
| **Overall survival** | **Median months** | 19.8 | 23.5 | 14.8 |
| | **≥ 1 year** | 67% | 76% | 59% |
| | **≥ 2 years** | 41% | 48% | 34% |
| **Age** | **Median years** | 57 | 57 | 56 |
| **Sex** | **% Male** | 78% | 84% | 73% |
| **Karnofsky status ≥ 80** | | 100% | 100% | 100% |
| **Time since initial tumor diagnosis** | **Mean years** | 3.4 | 2.4 | 4.3 |
| | **Median years** | 2.3 | 1.7 | 2.8 |
| | **< 1 year** | 25% | 29% | 21% |
| **Nephrectomy** | | 91% | 84% | 97% |
| **Prior cytokine therapy** | | 69% | 68% | 70% |
| **Other prior anti-tumor therapies** | **Chemotherapy** | 27% | 26% | 27% |
| | **Hormones** | 2% | 0% | 3% |
| | **Vaccines** | 3% | 3% | 3% |
| | **Investigational** | 2% | 0% | 3% |
| **MSKCC prognostic score** | **Favorable** | 41% | 39% | 42% |
| | **Intermediate** | 59% | 61% | 58% |
| **Metastatic sites** | **Lung** | 83% | 84% | 82% |
| | **Regional lymph nodes** | 25% | 23% | 27% |
| | **Distant lymph nodes** | 67% | 71% | 64% |
| | **Liver** | 34% | 36% | 33% |
| **No. of tumor lesions** | **1** | 8% | 10% | 6% |
| | **2** | 9% | 13% | 6% |
| | **≥ 3** | 83% | 77% | 88% |

**Supplementary Table 2: Baseline characteristics of patients stratified by immune responses (PP).**

| | | Immune responders (*n*=39) | Non-immune responders (*n*=22) | Multi-TUMAP responders (*n*=16) | Non-multi-TUMAP responders (*n*=45) |
|---|---|---|---|---|---|
| **Age** – years | mean | 57.4 | 58.6 | 50.9 | 60.3 |
| | median | 56 | 58 | 52.5 | 59 |
| | *P* | 0.693 | | 0.008 | |
| **Gender** – males | *n* (%) | 29 (74.4%) | 18 (81.8%) | 15 (93.8%) | 32 (71.1%) |
| | *P* | 0.508 | | 0.095 | |
| **Karnofsky status** – ≥80% | *n* (%) | 39 (100%) | 22 (100%) | 16 (100%) | 45 (100%) |
| **MSKCC risk group** | | | | | |
| Favorable | *n* (%) | 16 (41%) | 8 (36%) | 7 (44%) | 17 (38%) |
| Intermediate | *n* (%) | 23 (59%) | 14 (64%) | 9 (56%) | 28 (62%) |
| | *P* | 0.721 | | 0.675 | |
| **Time since initial tumor** | mean | 3.5 | 2.7 | 2.3 | 3.5 |
| **Diagnosis** – years | median | 1.9 | 2.0 | 1.5 | 2.5 |
| | *P* | 0.375 | | 0.208 | |
| **Time from prior therapy** | mean | 6.0 | 4.4 | 3.9 | 5.9 |
| **to Visit C** – months | median | 2.0 | 2.2 | 2.2 | 1.9 |
| | *P* | 0.492 | | 0.417 | |
| **Nephrectomy** – yes | *n* (%) | 36 (92.3%) | 19 (86.4%) | 16 (100%) | 39 (86.7%) |
| **Prior therapy** | | | | | |
| Cytokines | *n* (%) | 29 (74.4%) | 13 (59.1%) | 11 (68.8%) | 31 (68.9%) |
| | *P* | 0.220 | | 0.992 | |
| TKIs | *n* (%) | 13 (33.4%) | 10 (45.5%) | 7 (43.8%) | 16 (35.6%) |
| | *P* | 0.350 | | 0.562 | |
| TKI: sorafenib | *n* (%) | 7 (17.9%) | 1 (4.5%) | 2 (12.5%) | 6 (13.3%) |
| TKI: sunitinib[a] | *n* (%) | 7 (17.9%) | 9 (40.9%) | 5 (31.3%) | 11 (24.4%) |
| | *P* | 0.056 | | 0.596 | |
| **Prior radiotherapy** – yes | *n* (%) | 6 (15.4%) | 4 (18.2%) | 1 (6.3%) | 9 (20%) |

| | | Immune responders (*n*=39) | Non-immune responders (*n*=22) | Multi-TUMAP responders (*n*=16) | Non-multi-TUMAP responders (*n*=45) |
|---|---|---|---|---|---|
| **Other prior antitumor therapies** | | | | | |
| Chemotherapy | *n* (%) | 10 (25.6%) | 6 (27.3%) | 4 (25%) | 12 (26.7%) |
| Monoclonal antibodies | *n* (%) | 1 (2.6%) | 1 (4.5%) | 0 (0%) | 2 (4.4%) |
| Hormones | *n* (%) | 1 (2.6%) | 0 (0%) | 0 (0%) | 1 (2.2%) |
| Vaccines (BCG) | *n* (%) | 0 (0%) | 2 (9.1%) | 0 (0%) | 2 (4.4%) |
| **Metastatic sites (radiologist)** | | | | | |
| Liver | *n* (%) | 7 (17.9%) | 10 (45.5%) | 1 (6.3%) | 16 (35.6%) |
| Lung | *n* (%) | 28 (71.8%) | 14 (63.6%) | 13 (81.3%) | 29 (64.4%) |
| Lymph nodes | *n* (%) | 30 (76.9%) | 15 (68.2%) | 14 (87.5%) | 31 (68.9%) |
| **Total sum of target lesion diameters** – mm | mean | 112.0 | 138.2 | 101.8 | 128.8 |
| | median | 112.8 | 89.8 | 102.0 | 102.0 |
| | *P* | 0.288 | | 0.332 | |
| **Total number of lesions** – *n* | | | | | |
| | mean | 4.9 | 5.0 | 4.8 | 5.0 |
| | median | 5.0 | 4.5 | 5.5 | 5.0 |
| | *P* | 0.871 | | 0.810 | |
| **No. of locations of tumor lesions (central radiologist)** | 1 | 6 (15.4%) | 4 (18.2%) | 4 (25.0%) | 6 (13.3%) |
| | 2 | 15 (38.5%) | 6 (27.3%) | 5 (31.3%) | 16 (35.6%) |
| | >=3 | 18 (46.2%) | 12 (54.5%) | 7 (43.8%) | 23 (51.1%) |
| | *P* | 0.777 | | 0.380 | |
| **Baseline lymphocyte levels**[b] – 10E9 $l^{-1}$ | mean | 1.51 | 1.50 | 1.51 | 1.50 |
| | median | 1.31 | 1.34 | 1.30 | 1.31 |
| | *P* | 0.763 | | 0.942 | |

Notes: [a] Patient 59-001 received both, sunitinib and sorafenib, as previous therapies.
[b] Values from VC for +CY patients and from V1 for –CY patients.

**Supplementary Table 3: Baseline characteristics and survival of IMA901-202 patients after prior cytokine therapy in comparison to historic controls.**

| | | IMA901-202 Prior Cytokine All | IMA901-202 Prior Cytokine –CY | IMA901-202 Prior Cytokine +CY | TARGET Placebo arm | TARGET Sorafenib arm | RTKCC-0511-014 Sunitinib |
|---|---|---|---|---|---|---|---|
| **Sample size (*n*)** | | 40 | 20 | 20 | 452 | 451 | 63 |
| **Overall survival** | **Median months** | 19.8 | 15.8 | not reached (max. follow-up 33.1) | 15.2 | 17.8 | 16.4 |
| | **≥ 1 year** | 73.3% | 66.8% | 83.6% | ~59% | ~66% | ~61% |
| | **≥ 2 years** | 45.9% | 38.2% | 54.5% | ~34% | ~36% | <42% |
| **Age** | **Median years** | 57 | 59 | 56 | 59 | 58 | 60 |
| **Sex** | **% male** | 78% | 65% | 90% | 75% | 70% | 68% |
| **Karnofsky status ≥ 80** | | 100% | 100% | 100% | 98% | 98% | 100% |
| **Time since initial tumor diagnosis** | **Mean years** | 3.4 | 4.4 | 2.3 | 3.3[a] | 2.8[a] | 4.1 |
| | **Median years** | 1.7 | 2.6 | 1.3 | 1.9[a] | 1.6[a] | 1.7 |
| **Nephrectomy** | | 93% | 95% | 90% | 94% | 93% | 92% |
| **Prior cytokine therapy** | | 100% | 100% | 100% | 81% | 83% | 100% |
| **Other prior anti-tumor therapies** | **Chemotherapy** | 35% | 40% | 30% | 31%[ab] | 27%[ac] | NA |
| | **Hormones** | 3% | 5% | 0% | 7%[a] | 7%[a] | NA |
| **MSKCC prognostic score** | **Favourable** | 45% | 45% | 45% | 50% | 52% | 54% |
| | **Intermediate** | 55% | 55% | 55% | 49% | 48% | NA |
| **Metastatic sites** | **Lung** | 90% | 90% | 90% | 77% | 77% | 81% |
| | **Liver** | 28% | 20% | 35% | 26% | 26% | 16% |
| **No. of tumor lesions** | **1** | 5% | 10% | 0% | 14% | 14% | 18% |
| | **2** | 8% | 5% | 10% | 29% | 29% | 24% |
| | **≥ 3** | 88% | 85% | 90% | 57% | 57% | 59% |

[a] Based on the Nexavar® EPAR with n=384 in the sorafenib and n=385 in the placebo arm
[b] Pyrimidine analogues 18.7%, Vinca alkaloids 12.7%
[c] Pyrimidine analogues 15.6%, Vinca alkaloids 11.5%

Shown are IMA901-202 patients of the per-protocol group that were first line treated with cytokines. Historic control parameters were derived from the Nexavar® EPAR (EMEA/H/C/000690 -IA/00029/G http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000690/WC500027704.pdf, September 2011), Sutent® EPAR (EMEA/H/C/000687 -II/0028 http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000687/WC500057737.pdf, September 2011) and references 1-3.

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