**Supplementary Table S1: Daily calorie and macronutrient composition of the FMD regimen used in the NCT03340935 and NCT03454282 trials**

**Day (n) Allowed foods/beverages Kcal/day Macronutrient composition (%Kcal)**

1 **Breakfast:** one tea or non-caloric tisane at the choice of the  ~ 600 Carbohydrates: 52-56%

patient (without sugar) Proteins: 11-15%

**Lunch:** a dish of mixed vegetables (up to 300 grams), Lipids: 31-35%

including spinach, cabbage, cauliflower, broccoli, lettuce

or zucchini, consumed as raw, boiled or steamed (~72 Kcal),

together with a tablespoon (12 grams) of extra virgin olive

oil (118 Kcal); 100 grams of whole wheat breas (~224 Kcal)

**Dinner:** 150 grams of green salad (~30 Kcal) with a teaspoon

of extra virgin olive oil (~54 Kcal); 1 orange, 1 apple or 1 pear

(average weight 250 grams) (~100 Kcal).

2 **Breakfast:** one tea or non-caloric tisane at the choice at the ~300 Carbohydrates: 8-12%

choice of the patient (without sugar). Proteins: 12-16%

**Lunch:** 200 grams of green salad (~40 Kcal) with a teaspoon Lipids: 74-78%

of extra virgin olive oil (~54 Kcal). Alternatively, an equivalent

amount (~200 grams) of the following vegetables: spinach,

cabbage, cauliflower, broccoli, lettuce, zucchini, or 50 grams

of onions. Up to half a lemon per day.

**Dinner:** 20 grams of shelled walnuts (~180 Kcal) or 30 grams

of almonds (~180 Kcal).

3 **Breakfast:** one tea or non-caloric tisane at the choice at the ~300 Carbohydrates: 8-12%

choice of the patient (without sugar). Proteins: 12-16%

**Lunch:** 200 grams of green salad (~40 Kcal) with a teaspoon Lipids: 74-78%

of extra virgin olive oil (~54 Kcal). Alternatively, an equivalent

amount (~200 grams) of the following vegetables: spinach,

cabbage, cauliflower, broccoli, lettuce, zucchini, or 50 grams

of onions. Up to half a lemon per day.

**Dinner:** 20 grams of shelled walnuts (~180 Kcal) or 30 grams

of almonds (~180 Kcal).

4 **Breakfast:** one tea or non-caloric tisane at the choice at the ~300 Carbohydrates: 8-12%

choice of the patient (without sugar). Proteins: 12-16%

**Lunch:** 200 grams of green salad (~40 Kcal) with a teaspoon Lipids: 74-78%

of extra virgin olive oil (~54 Kcal). Alternatively, an equivalent

amount (~200 grams) of the following vegetables: spinach,

cabbage, cauliflower, broccoli, lettuce, zucchini, or 50 grams

of onions. Up to half a lemon per day.

**Dinner:** 20 grams of shelled walnuts (~180 Kcal) or 30 grams

of almonds (~180 Kcal).

5 **Breakfast:** one tea or non-caloric tisane at the choice of the  ~300 Carbohydrates: 43-47%

patient (without sugar). Proteins: 13-17%

**Lunch:** a dish of mixed vegetables (up to a total of 300 grams), Lipids: 38-42%

including spinach, cabbage, cauliflower, broccoli, lettuce or

zucchini, consumed as raw, boiled or steamed (~75 Kcal),

together with a tablespoon (12 grams) or extra virgin olive oil

(~108 Kcal). 1 orange, 1 apple or 1 pear (average weight 250

grams) (~100 Kcal).

**Dinner:** 100 grams of green salad (~20 Kcal) with a teaspoon

of extra virgin olive oil (~54 Kcal).

**Supplementary Table S2: minor and major deviations during the FMD in the NCT03340935 trial**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Patients** | | | **FMD Cycles** | | |
| **Total**  **N=101 (%)** | **Subgroups**  **N/Total Subgroup (%)** | | **Total**  **N=440 (%)** | **Subgroups**  **N/Total Subgroup (%)** | |
|  |  |  | |  |  | |
|  |  |  | |  |  | |
| Minor deviations | 23 (22.8) | Breast  Colorectal  Lung  Uterus  Pancreas  Melanoma | 16/56 (28.6)  2/10 (20.0)  2/7 (28.6)  1/1 (100)  1/3 (33.3)  1/3 (33.3) | 29 (6.6) | Breast  Colorectal  Lung  Uterus  Pancreas  Melanoma | 18/278 (6.5)  4/37 (10.8)  2/31 (6.5)  3/3 (100)  1/7 (14.3)  1/8 (12.5) |
|  |  |  |  |  |  |  |
| Major deviations | 6 (5.9) | Breast  Pancreas  Prostate  Colorectal | 3/56 (5.4)  1 /3 (33.3)  1/4 (25.0)  1/10 (10.0) | 7 (1.6) | Breast  Pancreas  Prostate  Colorectal | 4/278 (1.4)  1/7 (14.3)  1/18 (5.6)  1/37 (2.7) |
|  |  |  |  |  |  |  |
| Full compliance | 72 (71.3) |  |  | 404 (91.8) |  |  |
|  |  |  |  |  |  |  |

**Supplementary Table S3: cohort of 13 breast cancer patients treated with first-line cytotoxic ChT without the FMD in the context of the observational 79/17 study**

|  |  |  |  |
| --- | --- | --- | --- |
|  | | **Patient population**  **N = 13** | |
| **Characteristic** |  | **N** | **%** |
| **Age** (years) | ≥ 60  < 60 | 5  8 | 38.5  61.5 |
| **Sex** | Female | 13 | 100 |
| **ECOG PS** | 0  1 | 9  4 | 69.2  30.8 |
| **Tumor type** | TNBC  HR+ BC | 12  1 | 92.3  7.7 |
| **Disease stage** | Advanced | 13 | 100 |
| **Line of treatment** | First-line | 13 | 100 |
| **Type of chemotherapy** | Carboplatin-gemcitabine  Carboplatin-paclitaxel  Doxorubicin-cyclophosphamide | 10  2  1 | 76.9  15.4  7.7 |
| **BMI at enrollment** | ≥ 25 kg/m2  20-24.9 kg/m2 | 6  7 | 46.2  53.8 |
|  |  |  |  |

BMI: Body Mass Index; ECOG PS: Eastern Cooperative Oncology Group; HR+ BC: hormone receptor-positive breast cancer; TNBC: triple-negative breast cancer

**Supplementary Table S4: cohort of 38 cancer patients enrolled in the NCT03340935 trial, and undergoing PBMC evaluations before and after the FMD**

|  |  |  |  |
| --- | --- | --- | --- |
|  | | **Patient population**  **N = 38** | |
| **Characteristic** |  | **N** | **%** |
| **Age** (years) | ≥ 60  < 60 | 13  25 | 34.2  65.8 |
| **Sex** | Male  Female | 7  31 | 18.4  81.6 |
| **ECOG PS** | 0  1 | 28  10 | 73.7  26.3 |
| **Tumor type** | Breast  Lung  Colorectal  Prostate  Germinal  Chronic lymphocytic leukemia  Sarcoma  Kidney | 29  2  2  1  1  1  1  1 | 76.4  5.3  5.3  2.6  2.6  2.6  2.6  2.6 |
| **Disease stage** | Advanced  Localized/early stage | 30  8 | 78.9  21.1 |
| **Concomitant treatment** | Chemotherapy  Endocrine (± targeted therapies)  Best supportive care  Targeted therapy  Radiotherapy | 24  9  3  1  1 | 63.2  23.7  7.9  2.6  2.6 |
| **BMI at enrollment** | ≥ 25 kg/m2  20-25 kg/m2 | 20  18 | 52.6  47.4 |
|  |  |  |  |

**Supplementary Table S5: cohort of 13 advanced TNBC patients enrolled in the NCT03340935 trial, and undergoing PBMC evaluations before and after the FMD**

|  |  |  |  |
| --- | --- | --- | --- |
|  | | **Patient population**  **N = 13** | |
| **Characteristic** |  | **N** | **%** |
| **Age** (years) | ≥ 60  < 60 | 3  10 | 23.1  76.9 |
| **Sex** | Female | 13 | 100 |
| **ECOG PS** | 0  1 | 8  5 | 61.5  38.5 |
| **Tumour type** | TNBC | 13 | 100 |
| **Disease stage** | Advanced | 13 | 100 |
| **Line of treatment** | First-line | 13 | 100 |
| **Type of chemotherapy** | Carboplatin-gemcitabine | 13 | 100 |
| **BMI at enrollment** | ≥ 25 kg/m2  20-24.9 kg/m2 | 7  6 | 53.8  46.2 |
|  |  |  |  |

BMI: Body Mass Index; ECOG PS: Eastern Cooperative Oncology Group; TNBC: triple-negative breast cancer

**Supplementary Table S6: clinical characteristics of 22 patients enrolled in the DigesT trial (NCT03454282) and undergoing IHC and RNA-seq analysis of tumor tissues**

|  |  |  |  |
| --- | --- | --- | --- |
|  | | **Patient population**  **N = 22** | |
| **Characteristic** |  | **N** | **%** |
| **Age** (years) | ≥ 60  < 60 | 4  18 | 18.2  81.8 |
| **Sex** | Female | 22 | 100 |
| **ECOG PS**  **Menopausal status** | 0  Pre/peri  Post | 22  13  9 | 100  59.1  40.9 |
| **Tumor histology**  **Tumor sub-type** | Ductal  Lobular  Luminal A  Luminal B  HER2+ | 20  2  13  8  1 | 90.9  9.1  59.1  36.4  4.5 |
| **Pathologic stage (TNM)** | IA  IB  IIA  IIB  IIIA | 8  4  6  1  3 | 36.4  18.2  27.3  4.5  13.6 |
| **BMI at enrollment** | ≥ 25 kg/m2  20-24.9 kg/m2 | 11  11 | 50  50 |
|  |  |  |  |

**Supplementary Table S17: List of reagents and resources, including the source and Identification code, used for the immunological analyses of the NCT03340935**

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **SOURCE** | **IDENTIFIER** |
| Antibodies | | |
| Mouse monoclonal anti-human LAG3 (clone: 17B4) | AdipoGen | Cat# AG-20B-0012F, RRID:AB\_2490196 |
| Mouse monoclonal anti-human CD69 (clone: FN50) | BD Biosciences | Cat# 555531, RRID:AB\_395916 |
| Mouse monoclonal anti-human PD-1 (clone: PD1.3) | Beckman Coulter | Cat# A78885, RRID: not available |
| Mouse monoclonal anti-human CD8 (clone: RPA-T8) | BD Biosciences | Cat# 555369, RRID:AB\_398595 |
| Mouse monoclonal anti-human CD8 (clone: C8/14B) | Dako | Cat# M7103, RRID:AB\_2075537 |
| Mouse monoclonal anti-human CD8 (clone: SK1) | BioLegend | Cat# 344742, RRID:AB\_2566513 |
| Mouse monoclonal anti-human CD3 (clone: SK7) | BD Biosciences | Cat# 560176, RRID:AB\_1645475 |
| Mouse monoclonal anti-human CD3 (clone: UCHT1) | Beckman Coulter | Cat# B00068, RRID: not available |
| Mouse monoclonal anti-human CD45RA (clone: HI 100) | BD Biosciences | Cat# 560674, RRID:AB\_1727497 |
| Rat monoclonal anti-human TIM3 (clone: 344823) | R and D Systems | Cat# FAB2365N, RRID:AB\_10971286 |
| Rat monoclonal anti-human CCR7 (clone: 3D12) | BD Biosciences | Cat# 557734, RRID:AB\_396842 |
| Mouse monoclonal anti-human CD16 (clone: 3G8) | BD Biosciences | Cat# 555406, RRID:AB\_395806 |
| Mouse monoclonal anti-human CD3 (clone: B27) | Beckman Coulter | Cat# A07748, RRID:AB\_1575956 |
| Mouse monoclonal anti-human CD3 (clone: HT3alfa) | BD Biosciences | Cat# 555339, RRID:AB\_395745 |
| Mouse monoclonal anti-human CD25 (clone: M-A251) | BD Biosciences | Cat# 560503, RRID:AB\_1727453 |
| Mouse monoclonal anti-human CD4 (clone: SK3) | BD Biosciences | Cat# 562970, RRID:AB\_2744424 |
| Mouse monoclonal anti-human GZMB (clone: GB11) | BD Biosciences | Cat# 560211, RRID:AB\_1645488 |
| Mouse monoclonal anti-human FOXP3 (clone: 259D/C7) | BD Biosciences | Cat# 560046, RRID:AB\_1645508 |
| Mouse monoclonal anti-human IFNG (clone: B27) | BD Biosciences | Cat# 554702, RRID:AB\_398580 |
| Mouse monoclonal anti-human Ki67 (clone: B56) | BD Biosciences | Cat# 561277, RRID:AB\_10611571 |
| Mouse monoclonal anti-human CD14 (clone: RMO52) | Beckman Coulter | Cat# B92421, RRID: not available |
| Mouse monoclonal anti-human CD33 (clone: WM53) | BD Biosciences | Cat# 561160, RRID:AB\_10561683 |
| Mouse monoclonal anti-human CD11b (clone: ICRF44) | BD Biosciences | Cat# 562399, RRID:AB\_2737613 |
| Mouse monoclonal anti-human CD15 (clone: HI98) | BD Biosciences | Cat# 560828, RRID:AB\_10563612 |
| Mouse monoclonal anti-human HLA-DR (clone: G46-6) | BD Biosciences | Cat# 559866, RRID:AB\_398674 |
| Mouse monoclonal anti-human PD-L1 (CD274) (clone: MIH1) | BD Biosciences | Cat# 557924, RRID:AB\_647198 |
| Mouse monoclonal anti-human CD127 (clone: HIL-7R-M21) | BD Biosciences | Cat# 560822, RRID:AB\_2033938 |
| Mouse monoclonal anti-human CD56 (clone: NCAM16.2) | BD Biosciences | Cat# 563041, RRID:AB\_2732786 |
| Mouse monoclonal anti-human CD56 (clone: B159) | BD Biosciences | Cat# 562794, RRID:AB\_2737799 |
| Mouse monoclonal anti-human CD25 (clone: B1.49.9) | Beckman Coulter | Cat# B09684, RRID:AB\_2861133 |
| Chemicals | | |
| Ficoll-Paque gradient | GE Healthcare Life Sciences | Cat# 17144002 |
| BD GolgiStop | BD Biosciences | Cat# 554724 |
| BD Cytofix/Cytoperm | BD Biosciences | Cat# 554714 |
| Commercial Assays | | |
| LIVE/DEAD Fixable Green Dead Cell Stain Kit | ThermoFisher | Cat# L23101 |
| LIVE/DEAD Fixable Violet Dead Cell Stain Kit | ThermoFisher | Cat# L34955 |

**Supplementary Table S18. DigesT high dimensional flow cytometry antibody matrices**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | |  |  |  |
|  | **Matrix A** | | | | |
|  | **Marker** | **Fluorochrome** | **Clone** | **Source** | **Identifier** |
| 1 | LAG3 | FITC | 17B4 | Adipogen | Cat# AG-20B-0012F, RRID:AB\_2490196 |
| 2 | CD3 | PC5.5 | UCHT1 | Beckman Coulter | Cat# B49203 |
| 3 | CD4 | KrO | 13B8.2 | Beckman Coulter | Cat# A96417 |
| 4 | CD25 | APC | M-A251 | BD Biosciences | Cat# 555434, RRID:AB\_398598 |
| 5 | CD127 | PE | R34.34 | Beckman Coulter | Cat# B49220 |
| 6 | PD1 | PC7 | PD1.3.5 | Beckman Coulter | Cat# A78885 |
| 7 | CD28 | ECD | CD28.2 | Beckman Coulter | Cat# 6607111 |
| 8 | TIM3 | ALEXA 700 | 344823 | R and D Systems | Cat# FAB2365N, RRID:AB\_10971286 |
| 9 | CD56 | ALEXA 750 | N901 | Beckman Coulter | Cat# B46024 |
| 10 | CD8 | BV605 | SK1 | BioLegend | Cat# 344742, RRID:AB\_2566513 |
| 11 | CD16 | BV650 | 3G8 | BioLegend | Cat# 302042, RRID:AB\_2563801 |
| 12 | LIVE DEAD | Violet |  | ThermoFisher | Cat# L34955 |
|  |  |  |  |  |  |
|  | **Matrix B** | | | | |
|  | **Marker** | **Fluorochrome** | **Clone** | **Supplier** | **Identifier** |
| 1 | LIVE DEAD | FITC |  | ThermoFisher | Cat# L23101 |
| 2 | CD3 | ECD | B27 | Beckman Coulter | Cat# A07748, RRID:AB\_1575956 |
| CD19 | J3-119 | Beckman Coulter | Cat# A07770 |
| CD20 | B9E9 | Beckman Coulter | Cat# B92433 |
| CD56 | N901 | Beckman Coulter | Cat# B49214 |
| 3 | LOX-1 | PE | 15C4 | BioLegend | Cat# 358604, RRID:AB\_2562181 |
| 4 | CD33 | PC5.5 | D3HL60.251 | Beckman Coulter | Cat# B36289 |
| 5 | HLA-DR | APC | G46-6 | BD Biosciences | Cat# 559866, RRID:AB\_398674 |
| 6 | PDL-1 | PC7 | PD-L1 | Beckman Coulter | Cat# A78884 |
| 7 | CD14 | ALEXA 750 | RMO 52 | Beckman Coulter | Cat# B92421 |
| 8 | CD15 | KrO | 80H5 | Beckman Coulter | Cat# B01176 |
| 9 | CD11b | PB450 | Bear1 | Beckman Coulter | Cat# B36296 |
| 10 | CD16 | BV650 | 3G8 | BioLegend | Cat# 302042, RRID:AB\_2563801 |
|  |  |  |  |  |  |
|  | **Matrix C** | | | | |
|  | **Marker** | **Fluorochrome** | **Clone** | **Supplier** | **Identifier** |
| 1 | CD4 | FITC | RPA-T4 | BD Biosciences | Cat# 555346, RRID:AB\_395751 |
| 2 | CD3 | PC5.5 | UCHT1 | Beckman Coulter | Cat# B49203 |
| 4 | CCR7 | ALEXA 647 | 3D12 | BD Biosciences | Cat# 557734, RRID:AB\_396842 |
| 5 | CD45RO | PE | UCHL-1 | BD Biosciences | Cat# 347967, RRID:AB\_400362 |
| 6 | PD1 | PC7 | PD1.3.5 | Beckman Coulter | Cat# A78885 |
| 7 | CD27 | ECD | 1A4CD27 | Beckman Coulter | Cat# B26603 |
| 8 | CD45RA | APCH7 | HI100 | BD Biosciences | Cat# 560674, RRID:AB\_1727497 |
| 9 | CD8 | BV605 | SK1 | BioLegend | Cat# 344742, RRID:AB\_2566513 |
| 10 | LIVE DEAD | PB450 |  | ThermoFisher | Cat# L34955 |
|  |  |  |  |  |  |
|  | **Matrix D** | | | | |
|  | **Marker** | **Fluorochrome** | **Clone** | **Supplier** | **Identifier** |
| 1 | LIVE DEAD | FITC |  | ThermoFisher | Cat# L23101 |
| 2 | CD19 | ECD | J3-119 | Beckman Coulter | Cat# A07770 |
| CD20 | B9E9 | Beckman Coulter | Cat# B92433 |
| CD56 | N901 | Beckman Coulter | Cat# B49214 |
| 3 | VISTA | PE | 730804 | R and D Systems | Cat# FAB71261P |
| 4 | TIM3 | ALEXA 700 | 344823 | R and D Systems | Cat# FAB2365N, RRID:AB\_10971286 |
| 5 | HLA-DR | APC | G46-6 | BD Biosciences | Cat# 559866, RRID:AB\_398674 |
| 6 | CD3 | PC7 | UCHT1 | Beckman Coulter | Cat# 6607100 |
| 7 | CD14 | ALEXA 750 | RMO 52 | Beckman Coulter | Cat# B92421 |
| 8 | CX3CR1 | BV510 | 2A9-1 | BD Biosciences | Cat# 744487, RRID:AB\_2742267 |
| 9 | CD192 | BV421 | 48607 | BD Biosciences | Cat# 564067, RRID:AB\_2738573 |
| 10 | CD16 | BV650 | 3G8 | BioLegend | Cat# 302042, RRID:AB\_2563801 |