

control study population. It implies that the study population may be heterogeneous, including patients who have very different tumor burdens and disease characteristics. Patients may enter the study with measurable disease, evaluable disease or no evidence of disease (i.e. post surgery). The inclusion of the latter two groups is justified since these groups were included in our historical control and can be evaluated for disease progression or relapse. The patients will be enrolled on this study with the intention to approximate the various percentages of patient subgroups with the most significant differences in prognostic value found in the 96-C-0104 study. The hormone receptor status has prognostic significance and patients will be stratified by HR status. Patients with metastases to the bone only have a longer progression free survival and patients with three or more organs involved with metastatic disease have a shorter survival. Care will be taken during patient recruitment to approximate the percentages of these two subgroups in our historical study. Residual variability in the two patient populations can be addressed at the time of the statistical analysis by appropriate corrections in the Cox regression model (see statistical section (5.4.2).

Finally, consideration has been given to the use of corticosteroids and their possible confounding effects during this protocol. Their use will be expressly limited to premedication for Paclitaxel administration and as an adjunct anti-emetic at the time of the high-dose chemotherapy. The initial vaccination will take place ten days to two weeks before the first dose of Dexamethasone and patients will undergo lymphocyte collection before the onset of chemotherapy and, therefore, administration of Dexamethasone. The regimen of Melphalan-Etoposide will be overwhelmingly responsible for the immunosuppression so that the addition of Dexamethasone as adjunct anti-emetic should not be of significance provided that the treatment is stopped at day -2 before stem cell transplantation.

3.1.2. Overview

The patient population (defined in section 2) will consist of patients with metastatic breast cancer (stage IV), CEA positive, hormone receptor positive or negative. Forty patients (40) with Hormone Receptor positive tumors and twenty-three (23) patients with Hormone Receptor negative tumors will be enrolled on this study (see section 5.4 for calculation of the sample size).

The patients will receive intensive treatment for their disease including: induction chemotherapy, high-dose chemotherapy / transplantation followed by irradiation and hormonal therapy as indicated. This will be referred to as the "Concurrent Therapy" in the rest of the protocol. The study will evaluate patient clinical response to series of immunizations with two different poxvirus-based recombinant vaccines:

- Recombinant **Vaccinia**-CEA-Tricom (rV-CEA(6D)/TRICOM, Therion Biologics)
- Recombinant **Fowlpox**-CEA-Tricom (rF-CEA(6D)/TRICOM, Therion Biologics)
- Initial immunization will be given before any chemotherapy on the protocol. It will consist of a single subcutaneous injection of rV-CEA(6D)/TRICOM.
- Lymphopheresis will take place ten to fourteen days later.
- "Concurrent Therapy" will then take place: chemotherapy followed by high-dose chemotherapy / transplantation and, if clinically indicated, irradiation. Following transplantation, hormone receptor positive patients will be treated with hormonal therapy as clinically appropriate.
- Re-immunizations will start immediately after the end of all chemotherapy. It will consist of:

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- an “immediate” re-immunization with the rF-CEA(6D)/TRICOM on day +3 post transplantation
- An “early” series of two monthly rF-CEA(6D)/TRICOM re-immunizations to start 3 months post transplantation (after the end of radiation therapy) in conjunction with lymphocyte re-infusions and low dose IL-2.
- Four subsequent rF-CEA(6D)/TRICOM re-immunizations, 6 months apart:
 - “intermediate” at 10 months,
 - “late 1” at 16 months,
 - “late 2” at 22 months and
 - “late 3” at 28 months post transplantation.

3.1.3. Nomenclature of treatment phases

The treatment consisting of “conventional” breast cancer therapy (chemotherapy, high-dose chemotherapy and subsequent radiation and hormonal therapies, if indicated) will be referred to as “**Concurrent Therapy Phase**”. Each cycle of chemotherapy will be named: -“**TC-cycle 1, 2 ...5**” for the Paclitaxel / Cyclophosphamide regimen, -“**AC-cycle 1...4**” for the Doxorubicin /Cyclophosphamide regimen. Each day of each cycle will be numbered as well. -“**Transplantation cycle**”: the numbering of days after transplant will start as transplant day is day 0 and will continue until patients are off study.

The treatment consisting of immunizations will be referred to as “**Immunization Phase**”. The pre-chemotherapy rV-CEA(6D)/TRICOM vaccination will be called “**rV-vaccine**”. The “immediate” post-transplantation rF-CEA-Tricom vaccination will be called “**rF1**”. The rF-CEA(6D)/TRICOM immunizations following lymphocyte infusions will be called “**rF 2** and **rF3**” respectively and the subsequent re-immunizations “**rF4**” through “**rF7**”.

3.1.4. Biological end-points

3.1.4.1. The assays

- **Class I response to CEA:** Elispot for peripheral lymphocyte γ -IFN production in response to a CEA peptide (CAP-1) in HLA-A2 patients⁽⁷⁴⁾.

CAP-1 is a 9-mer peptide (YLSGANLNL) of CEA which binds to HLA A2 and can trigger the generation of CEA-specific T-cells in patients immunized with rV-CEA and ALVAC-CEA. This CEA-specific response can only be evaluated in patients with the HLA A2 genotype (which is present in about 40-50% of the Caucasian and 20-30% of the African American population).

Methods: 96-well milliliter HA plates (Millipore Corporation, Bedford, MA) are coated with 100 μ l/well of capture MoAb against human γ -IFN at a concentration of 10 μ g/ml for 12h at RT. Plates are blocked for 30 min with RPMI 1640 plus 10% human AB serum. 1×10^5 PBMC are added to each well. CAP-1(6D) pulsed C1R-A2 cells are added to each well as antigen presenting cells (APC) at an effector:APC ratio of 1:1. Unpulsed C1R-A2 cells are used as a negative control. HLA-A2 binding Flu Matrix peptide 59-66 is used as a positive peptide control. Cells are incubated for 24h and lysed in phosphate buffered saline (PBS)-Tween (.05%). Biotinylated anti γ -IFN antibody diluted to 2 μ g/ml in PBS-Tween containing 1%bovine serum albumin (BSA) is added and incubated overnight in 5% CO₂ at 37°C. Plates are washed 3 times and developed with avidin alkaline phosphatase (GIBCO/BRL, Grand Island, NY) for 2h.

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