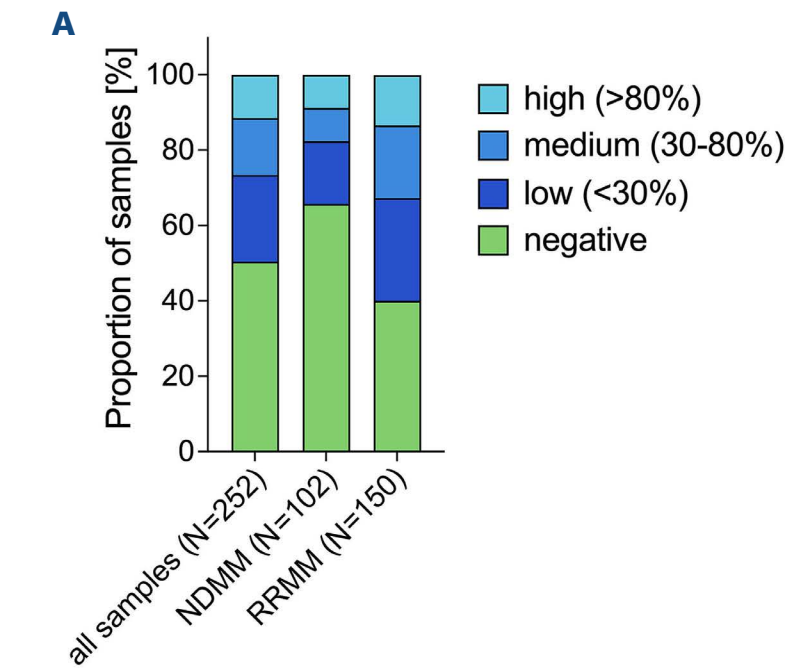
A phase I study of MAGE-A1-targeted T1367 T-cell receptor-based cell therapy in patients with advanced multiple myeloma

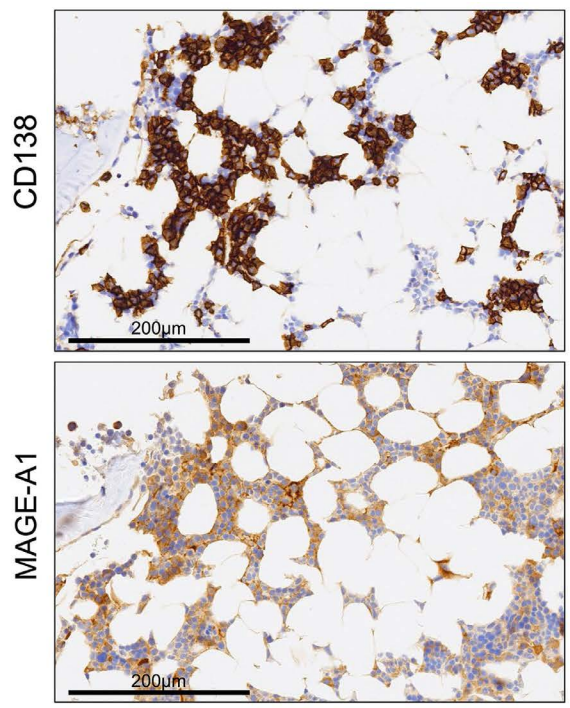
Cancer/testis (C/T) antigens are genes whose expression is silenced in healthy adult tissues except for male germ cells. Because C/T antigens are expressed in various cancers, they are potential targets for targeted therapies.[1] MAGE-A1, the first identified C/T antigen, was characterized as the target of an autologous cytotoxic T-cell clone recognizing the melanoma cell line MZ2E.[2] MAGE-A1 expression was described in biopsies from patients with multiple myeloma (MM),[3] an aggressive plasma cell malignancy, and was associated with poor prognosis in one study.[4] Despite recent advances in the treatment of MM, such as chimeric antigen receptor (CAR)T-cell therapy and bispecific T-cell engagers (BiTE),[5] MM remains incurable, and antigen loss has been described as resistance mechanism for immunotherapies.[6] Therefore, there is a high need for new treatment options and therapeutic targets. T-cell receptor (TCR) therapy represents another T-cell-based immunotherapeutic approach in cancer.[7] TCR T-cell therapy allows to target intracellular proteins presented by major histocompatibility (MHC) class I molecules with a higher antigen sensitivity compared to CAR T cells.[8,9] We have previously described TCR-T1367 with optimal affinity against MAGE-A1 for development of TCR-based cellular immunotherapy.[10] In this study, we present the analysis of a large cohort of MM patients for MAGE-A1 expression and its association with specific clinical and disease characteristics. Furthermore, we describe TCR-1367 T-cell production in an academic setting, and present clinical data from the phase I study (EudraCT: 2017-001208-30) investigating TCR-1367 T cells in two patients.

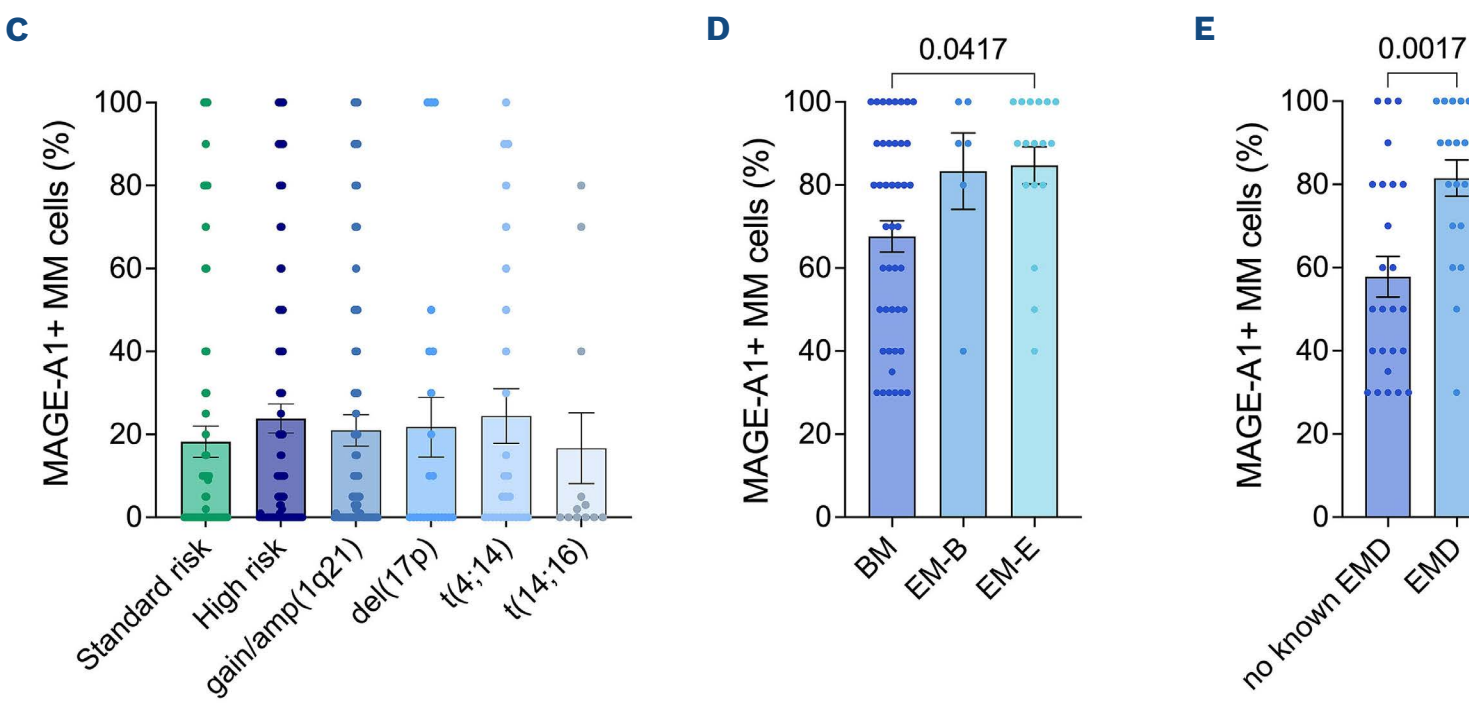
MAGE-A1 expression was investigated by immunohistochemistry (IHC) in 252 formalin-fixed, paraffin-embedded histological samples from 213 patients, collected from 2012 to 2022, using the commonly used anti-MAGE-A1 antibody MA454. The study was approved by the ethical committee of Charité-Universitätsmedizin Berlin (EA4/133/23). Statistical tests included Mann-Whitney test for comparing two categories and Kruskal-Wallis test for more than two categories. Clinical and genetic characteristics of our cohort are presented in the *Online Supplementary Table S1*. Out of the 252 samples, 27% presented with ≥30% MAGE-A1 positive (MAGE-A1$^+$) MM cells, 23% with a lower fraction and 50% without MAGE-A1 expression (Figure 1A). An exemplary slide of MAGE-A1 expressing MM cells is shown in Figure 1B. The fraction of MAGE-A1$^+$ samples (≥30% of MAGE-A1$^+$ MM cells), increased from 18% at diagnosis to 33% during relapse (Figure 1A) with a significant increase of the mean proportion of MAGE-A1$^+$ MM cells in all samples (15% *vs*. 26%; *P*=0.0002). For 131 patients cytogenetics and fluorescence *in situ* hybridization (FISH) results were available, with 59 classified as standard-risk and 72 as high-risk based on cytogenetic aberrations defined as del(17p), t(4;14), t(14;16), gain or amplification (1q21).[11,12] High-risk patients presented with a slightly higher proportion of MAGE-A1$^+$ samples (31%) compared to the standard-risk group (23%), but without significant difference in mean MAGE-A1 expression (24% *vs*. 18%; *P*=0.0788; Figure 1C).

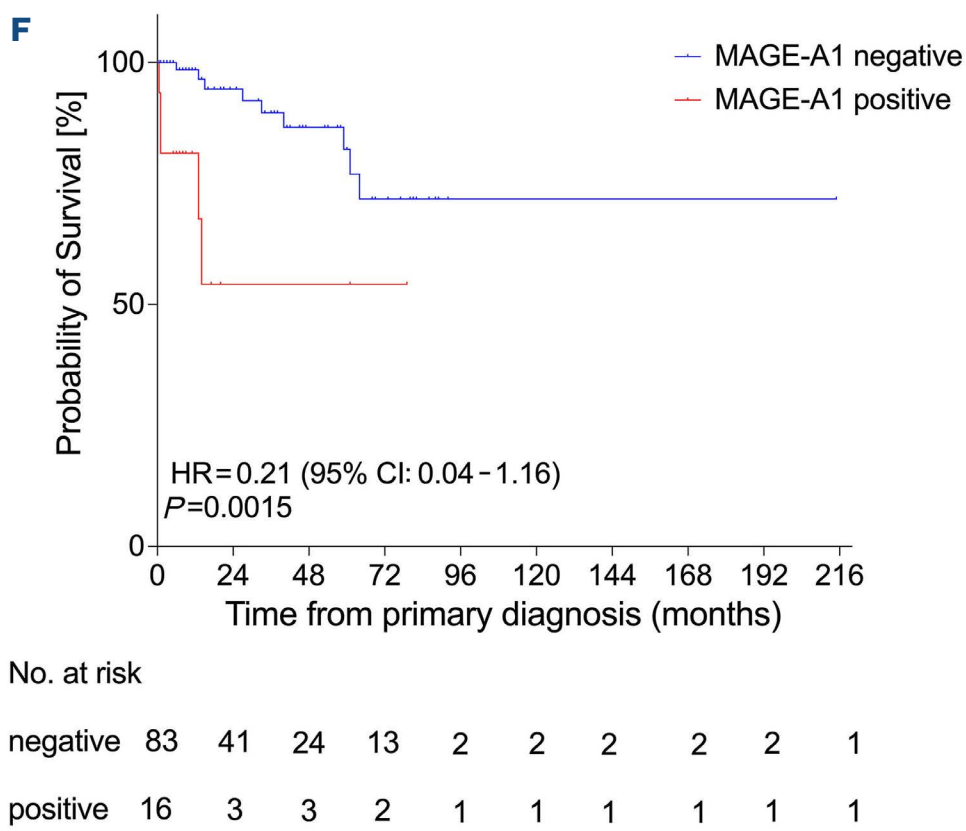
Furthermore, we investigated the association of MAGE-A1 expression with extramedullary disease (EMD). In bone marrow (BM) samples, 22% had ≥30% MAGE-A1$^+$ cells, compared to 55% in bone-related extramedullary myeloma (EM-B) and 46% in extraosseous extramedullary myeloma (EM-E) samples. The mean proportion of MAGE-A1$^+$ MM cells in positive samples was higher in EM-B (83%; *P*=0.3918) and EM-E (85%; *P*=0.0417) samples compared to BM (68%) as shown in Figure 1D. A significant higher proportion of MAGE-A1$^+$ MM cells in positive BM samples were found in patients with documented EMD compared to patients without EMD (82% *vs*. 58%; *P*=0.0017; Figure 1E). Analyzing 11 matched EMD and BM samples collected from the same time point and same patient we found only a weak correlation between the proportion of MAGE-A1$^+$ cells between these samples (R^2=0.3883; *P*=0.0405). Survival data were available for 99 newly diagnosed MM patients of which 83 were MAGE-A1-negative (<30% MAGE-A1$^+$ MM cells) and 16 positive (≥30% MAGE-A1$^+$ MM cells). Kaplan-Meier survival analysis revealed that MAGE-A1 expression at diagnosis was associated with impaired overall survival (OS) resulting in 2-year survival rates of 95% for negative and 54% for positive patients (median OS not-reached for both; hazard ratio [HR]=0.21; 95% confidence interval [CI]: 0.04-1.16; *P*=0.0015, log-rank test; Figure 1F).

To investigate MAGE-A1 as a therapeutic target, we applied the MAGE-$A1_{278-286}$- epitope-directed TCR-T1367 sequence[10] and transduced autologous T cells from patients with MM with retrovirus encoding TCR-T1367. TCR-1367 T cells were manufactured at Zellkulturlabor für Klinische Prüfung (ZKP), the GMP Facility of the Experimental and Clinical Research Center (ECRC), Charité-Universitätsmedizin Berlin. An overview of the manufacturing process is shown in Figure 2A. The manufacturing process was validated in three healthy donor validation runs. TCR-1367 T cells were manufactured for three patients (patients 001, 004,









No. at risk

negative	83	41	24	13	2	2	2	2	2	1
positive	16	3	3	2	1	1	1	1	1	1

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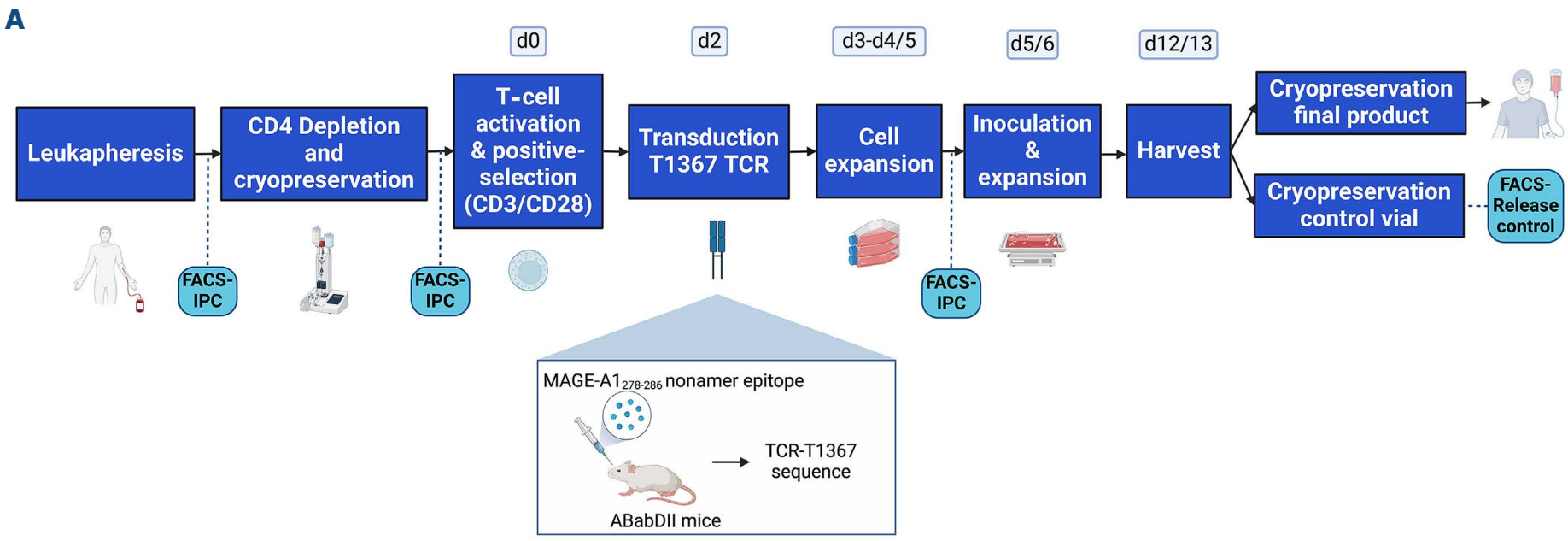
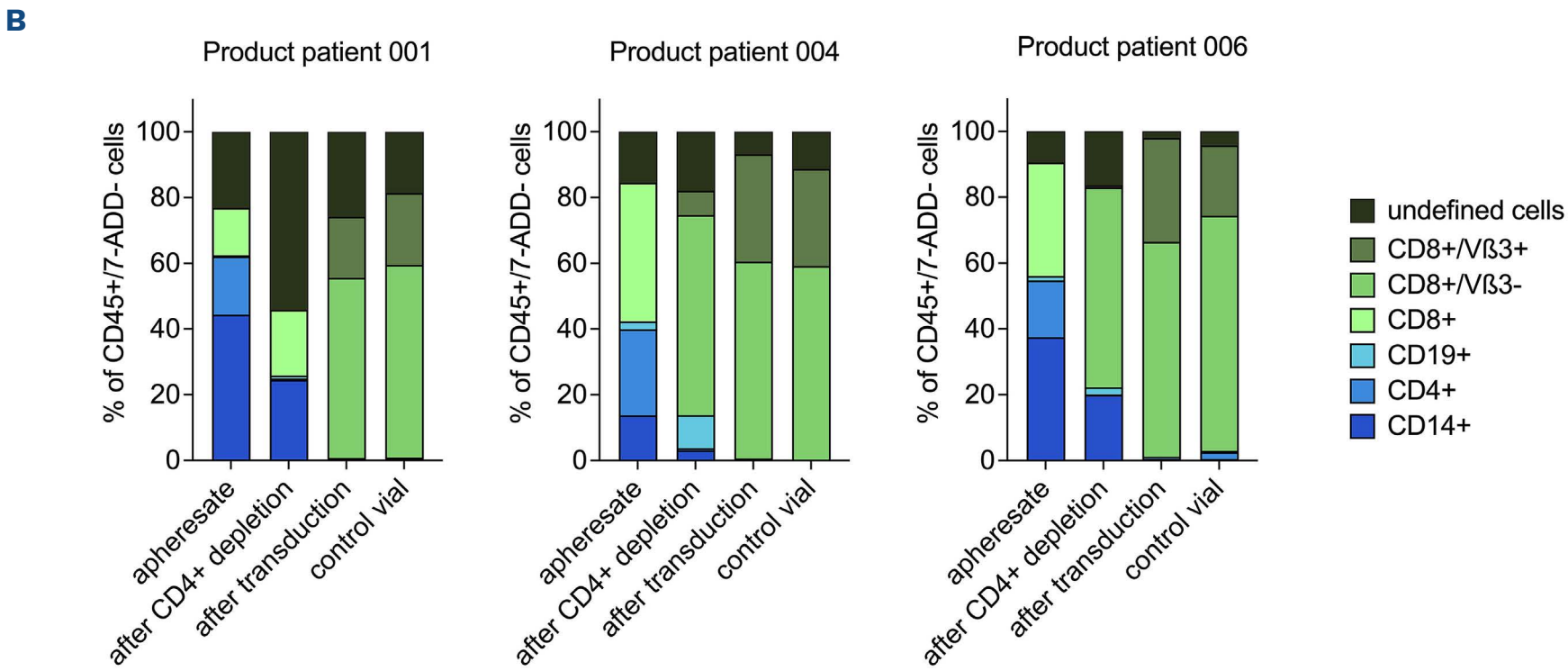
Figure 1. MAGE-A1 expression in multiple myeloma patient samples is associated with extramedullary disease and is a risk factor for overall survival. (A) Samples were classified according to the fraction of MAGE-A1-positive (MAGE-A1$^+$) multiple myeloma (MM) cells (negative; low =1-29%; medium =30-80%; high =>80%). Proportion of the different categories of MAGE-A1 expression shown for all samples, newly diagnosed MM (NDMM) and relapsed/refractory MM (RRMM). (B) Representative immunohistochemistry staining of MM bone marrow samples with monoclonal antibody MA454 and anti-CD138 of a patient with NDMM. BM is infiltrated with 30% of MM cells of which overall 10-20% are MAGE-A1$^+$. (C) Proportion of MAGE-A1$^+$ MM cells categorized according to the patients cytogenetics results. Mean and standard error of the mean shown. Kruskal-Wallis test. (D) Proportion of MAGE-A1$^+$ MM cells depending on sample type in MAGE-A1$^+$ samples (at least 30% positive MM cells). (E) Proportion of MAGE-A1$^+$ MM cells depending on the presence of extramedullary disease at any time of disease course in MAGE-A1$^+$ bone marrow samples. (F) Kaplan-Meier curves of patients with MAGE-A1 expression (at least 30% positive myeloma cells) and without (<30% positive myeloma cells) at time point of NDMM. HR: hazard ratio; CI: confidence interval.

006). T1367 expresses Vβ3, which was used as marker for T1367-transduced cells. The transduction rates, estimated by flow cytometry detection of Vβ3$^+$/CD8$^+$ cells 4 days after transduction, were 18.6% (001), 32.7% (004) and 31.6% (006) of all CD45$^+$/7-ADD$^-$ cells, and were stable throughout the freezing and thawing process. Only the product from patient 006 experienced a 10% decrease (Figure 2B). For patients 004 and 006 a sufficient cell proliferation after transduction was observed, reaching proliferation rates of 57-fold and 13-fold on day 12 (Figure 2C). In contrast, product 001 showed a proliferation rate of only 2-fold on day 12 (Figure 2C). We observed a strong correlation between the proliferation rate from day 2 to day 5 and the transduction rate in the final product (R^2=0.9441; *P*=0.0012; Figure 2D). The cell viability after thawing, determined by trypan blue staining, was 76.0% (001), 97.5% (004) and 93.5% (006). Due to the proliferation and viability data, the TCR-1367 T cells (patient 001) were not considered for therapy.

To evaluate safety and efficacy of TCR-1367 T cells, we conducted a one-armed, single-center, open-label, phase I clinical trial (EudraCT: 2017-001208-30). The summarized study design is shown in Figure 3A. The main inclusion criteria were age ≥18 years, relapsed and/or refractory disease requiring therapy, at least three prior lines of therapy, HLA-A*02:01 genotype, and at least 30% of MAGE-A1$^+$ MM cells assessed by IHC. The primary objective was to evaluate the safety and tolerability of TCR-1367 T cells. It was planned to enroll 12 patients in four cohorts with ascending doses of TCR-1367 T cells (10^5; 10^6; 10^7 and $5x10^7$ cells/kg body weight [BW] ± 20%). However, based on limited recruitment potential upon availability of BCMA-CAR T cells, and competing clinical studies investigating BiTE, the trial was closed by the sponsor after treating two patients (patients 004 and 006). All patients provided written informed consent and the trial was approved by the local ethical committee in Berlin, Germany (17/0259-EK13). The study was conducted in accordance with principles of good clinical practice and the Declaration of Helsinki. The patient characteristics are shown in *Online Supplementary Table S2*. Both patients were treated in the first dosing cohort and were eligible for safety and efficacy analysis. The time between apheresis to application of TCR-1367 T cells was 64 days (004) and 55 days (006). Altogether, we observed 18 treatment-emergent adverse events (TEAE) in the two patients with 11 classified as possibly study treatment-related. Four of the TEAE were CTCAE (version 4.03)[13] grade 3 or 4 and two were serious AE (febrile neutropenia and cancer pain), both likely related to chemotherapy and disease progression, respectively. All AE are listed in *Online Supplementary Table S3*. The best response according to the IMWG criteria[14] was minimal response for patient 004, while patient 006 experienced progressive disease. The time to next treatment was 110 days for patient 004 and 64 days for patient 006. In patient 004, the proportion of MAGE-A1$^+$ MM cells subsequently decreased from 80% to 60% to 30% 3 months after administration of TCR-1367 T cells (Figure 3B). MM cell infiltration decreased from 60% to 40%. However, the fraction of MAGE-A1$^+$ MM cells started to rise again 7 months after the administration of TCR-1367 T cells, reaching 40% (Figure 3B). As shown in Figure 3C for patient 006, no measurable effect of the TCR-1367 therapy on the MAGE-A1 expression was found. Patient 004 achieved a complete response under following BiTE treatment and is still alive 35 months after administration of TCR-1367 T cells. Patient 006 further progressed and died 3 months after receiving the study treatment from myeloma progression and disease-related pancytopenia with an infection of unknown focus, considered not related to the study treatment, resulting in an OS of 91 days. No TCR-1367 T cells could be detected by flow cytometry or quantitative polymerase chain reaction (qPCR) in pharmacokinetics samples, possibly due to the low cell number administered.

MAGE-A1, identified as a frequently expressed antigen in MM in our diagnostic study, could emerge as a valuable new target, especially considering loss of commonly targeted antigens like BCMA and GPRC5D under current therapies.[6] MAGE-A1 expression was associated with EMD and lower OS, aligning with findings in other MM patient cohorts.[3,15] In the phase I clinical trial investigating MAGE-A1-directed TCR-1367 T cells, we treated only two patients due to premature closure of the trial by the sponsor, making it impossible to provide conclusive safety and efficacy data. While we observed no severe TEAE, durable responses were not achieved, possibly due to the low dose of TCR-1367 T cells administered in the first dose cohort ($1x10^5$ cells/kg BW).





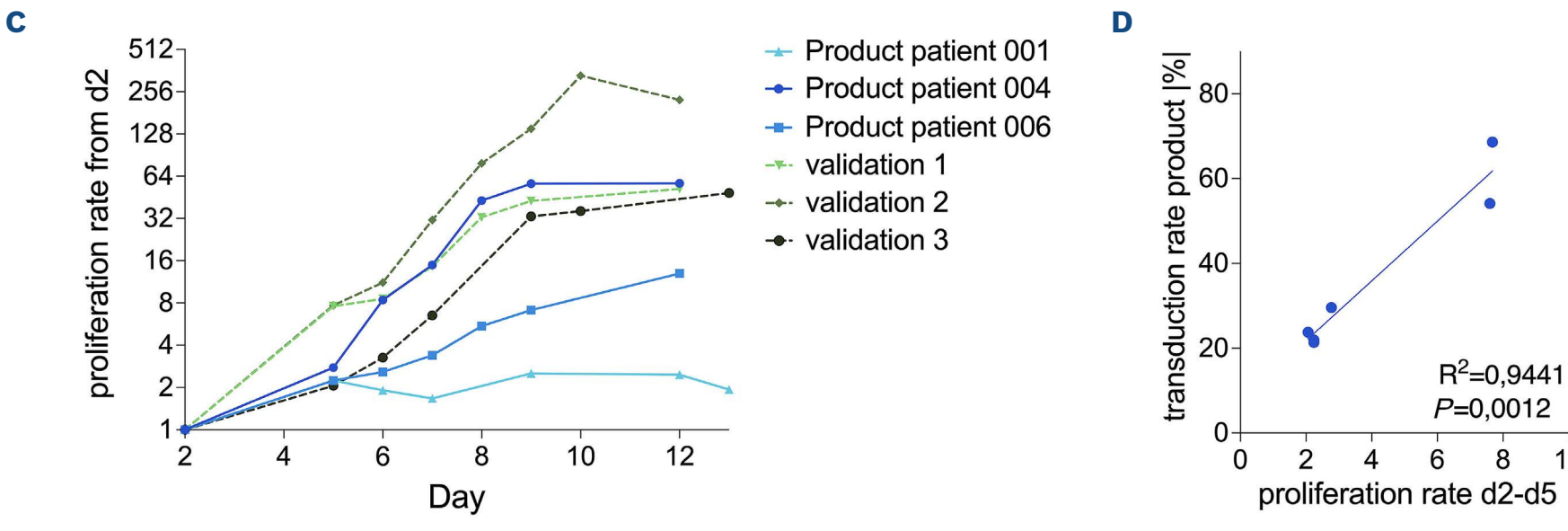


Figure 2. Production of TCR-1367 T cells and product characteristics. (A) Flow chart of manufacturing process of MAGE-A1-directed TCR-1367 T cells. TCR-T1367 sequence was isolated from ABabDII mice (transgenic for the human *TCR*α/β gene loci and human leukocyte antigen HLA-A*02:01) vaccinated with the MAGE-A1-derived nonamer epitope MAGE-A1$_{278-286}$. IPC: in process control. Created with BioRender.com. (B) Fluorescence-activated cell sorting (FACS) results for the 3 patients' cell products at different time points during manufacturing process. Data shown is pregated for CD45^{+}/7-ADD^{-} cells. (C) Proliferation rate of living cells from day 2 to harvest (day [d] 12/13), proliferation normalized to cell number from day 2. Data shown from the 3 patients' cell products and 3 validation runs. (D) Correlation of proliferation rate from day 2 to day 5 with transduction rate of final cell product from the 3 patients' cell products and 3 validation runs with simple linear regression.

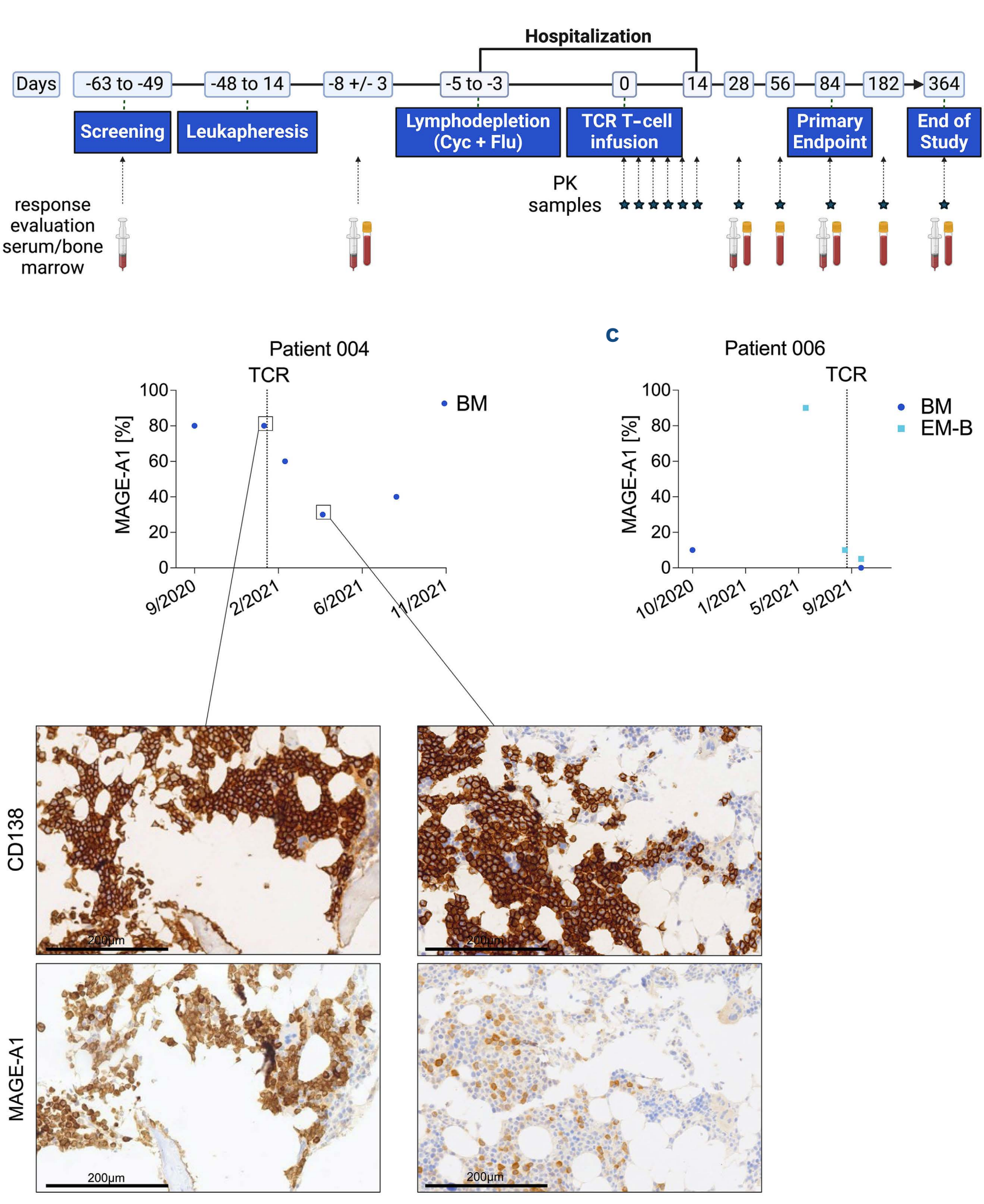


Figure 3. Phase I clinical trial of MAGE-A1-directed TCR-1367 T cells. (A) Study design of the phase I clinical trial with scheduled sample collection for myeloma response evaluation and pharmacokinetic (PK) analysis. Created with BioRender.com. (B) Time-coursed MAGE-A1 expression of myeloma cells of clinical trial patient 004 before and after the TCR-1367 T-cell administration in bone marrow. Representative immunohistochemistry staining (CD138, MAGE-A1) of sample before (left) and after (right) the TCR-1367 T-cell administration. Before myeloma infiltration is overall 60%, shown is an area with higher myeloma cell infiltration with 80% of cells positive for MAGE-A1. The after sample has 40% myeloma cell infiltration with a decrease to 30% of MAGE-A1-positive myeloma cells. (C) Time-coursed MAGE-A1 expression of myeloma cells of clinical trial patient 006 before and after the TCR-1367 T-cell administration in bone marrow (BM) and bone-associated extramedullary (EM-B) samples. TCR: T-cell receptor.

In conclusion, MAGE-A1 is an antigen expressed by a subset of MM patients associated with advanced disease and EMD. MAGE-A1-directed TCR-1367 therapy appears feasible for the tested dose in this patient group. Further clinical studies are required within the multi-refractory patient population, especially those relapsing after currently approved T-cell redirecting therapies.

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Disclosures

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Contributions

JK collected and analyzed the IHC expression data and created the graphs. IA, KJ, JB and A-CB provided the immunohistochemistry slides of MAGE-A1 expression. MO, AP, TB, UK, J K, IWB, AB and AN designed the study. MO, DH, VS, GW, TB and AP contributed the preliminary work for TCR-1367 and designed the manufacturing process with MV and HR. MV and HR manufactured the TCR-1367 T cells. MO, TB, AP, VS and EK designed the clinical trial. JK and AN wrote the manuscript. All authors revised the manuscript and approved the final version which was submitted.

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Data-sharing statement

Reasonable requests for further data will be considered. Requests should be directed to the corresponding authors.

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