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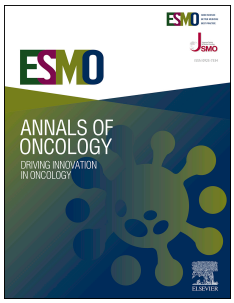

Neoadjuvant Intralesional Targeted Immunocytokines (Daromun) in Stage III Melanoma.

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Neoadjuvant Intralesional Targeted Immunocytokines (Daromun) in Stage III Melanoma.

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Highlights:

- PIVOTAL is the first phase 3 trial to demonstrate a statistically significant clinical benefit of neoadjuvant intralesional immunotherapy in stage III melanoma
- Daromun consists of two immunocytokines that selectively bind to a TME antigen and sequester the stimulatory agents at the disease site
- Neoadjuvant intralesional Daromun in patients with locally advanced, mostly pretreated melanoma results in 41% reduction in risk of recurrence or death
- The reduction in risk of distant metastases with intralesional Daromun suggests an induced systemic antitumor immune response
- Neoadjuvant Daromun may be a well-tolerated addition to the armamentarium for resectable locally advanced melanoma

ABSTRACT

Background

This phase 3 trial assessed daromun, a combination of two fibronectin-targeting immunocytokines (L19IL2 and L19TNF), as a neoadjuvant treatment for patients with clinically detectable stage IIIB/C melanoma (AJCC version 7).

Methods

Patients were randomized to 4 weekly intralesional daromun administrations (13 Mio IU of L19IL2 and 400 μg of L19TNF) followed by surgery, or upfront surgery. Pretreatment with approved adjuvant agents was allowed. The primary endpoint was recurrence-free survival (RFS): events were disease recurrence or death from any cause after complete surgical tumor resection.

Key findings

246 patients were randomized and included in the intention-to-treat analysis: 74% had undergone ≥ 2 prior surgical resections and 35% had received prior systemic therapy. At a median follow-up of 21 months, the neoadjuvant group (N=122) had a significantly longer RFS than the upfront surgery group (N=124), with a median RFS of 16.7 and 6.8 months, respectively (HR 0.59; 95% CI 0.41 to 0.86, p=0.005, log-rank test). The risk of distant recurrence was reduced by 40% in the neoadjuvant arm (HR=0.60; 95% CI [0.37; 0.95]; p=0.029). Grade ≥ 3 treatment-related adverse events (TRAEs) were 6.7% in the surgery alone arm and 27.1% in the daromun arm, mostly injection site reactions.

Conclusions

Neoadjuvant daromun resulted in a significantly longer RFS than upfront surgery in patients with locally advanced melanoma. TRAEs were transient and manageable. Neoadjuvant daromun is a new therapeutic option for patients with stage III melanoma, including those with locoregional recurrence after surgery and previous adjuvant therapy.

(ClinicalTrials.gov NCT02938299).

Keywords: Neoadjuvant, melanoma, resectable, locally advanced, targeted immunocytokines, immunotherapy, intralesional

INTRODUCTION

Recent studies [1, 2] have demonstrated a role for systemic neoadjuvant immunotherapy in stage III melanoma.[3, 4] In the randomized phase 2 SWOG-1801 trial, the neoadjuvant-adjuvant pembrolizumab group showed significantly longer event-free survival (EFS; HR: 0.58, 95% CI [0.39, 0.87]; P = 0.004) at a median follow-up of 14.7 months than the adjuvant-only group[1]. In the phase 3 NADINA trial, at a median follow-up of 9.9 months, the estimated 12-month EFS was 83.7% in the neoadjuvant ipilimumab + nivolumab group and 57.2% in the adjuvant group (HR:0.32, 99.9% CI [0.15, 0.66])[2]. Both studies specifically enrolled patients with clinically detectable stage III (NADINA) or stage III/IV (SWOG-1801) resectable melanoma at their initial presentation or at the time of the first detected metastases and no prior exposure to systemic immunotherapies (e.g., anti-CTLA-4, PD-1 or PD-L1) or targeted agents (BRAF and/or MEK inhibitors).

Neoadjuvant intralesional therapy with talimogene laherparepvec (T-VEC), an oncolytic genetically engineered herpes simplex virus type 1 that produces granulocyte-macrophage colony-stimulating factor was evaluated in resectable stage IIIB to IVM1a melanoma. In a randomized phase 2 study (NCT02211131), at a median follow-up of 63.3 months, the 5-year RFS was 22.3% in arm 1 (neoadjuvant T-VEC plus surgery) and 15.2% in arm 2 (surgery alone) (HR, 0.76; 80% CI, 0.60-0.97).[5]

Daromun, the combination of L19IL2 and L19TNF, has been studied in locally advanced melanoma: its mechanism of action is based on the specific activities of the two cytokines, each fused to L19, a fully human antibody fragment that binds to the alternatively spliced extra-domain B (EDB) of fibronectin (FN),[6] a known marker of neo-angiogenesis.[7, 8] A moderate to strong, diffuse expression of EDB FN can be observed in the majority of metastatic melanoma lesions. Conversely, a weak expression of EDB characterizes most primary melanoma lesions, and no expression of such isoform is detected in normal skin. [9] Antibody-cytokine fusions with the L19 moiety are preferentially anchored to the tumor stroma:[10, 11] their residence time in the injected lesions is much longer (≥ 72 hours) than that of non-targeted cytokines (<24 hours). [11]

In a phase 2 study in 22 patients with unresectable stage III-IVM1a melanoma, intralesional daromun resulted in a high proportion of objective responses even in non-injected melanoma lesions (OR=69%, CR=54%), suggesting a local and systemic immune response.[12]

Here we report the results of the phase 3 PIVOTAL trial, which evaluated the efficacy and safety of neoadjuvant intralesional daromun followed by surgery versus upfront surgery, in patients with resectable locally advanced melanoma, most of whom had recurred after prior

surgery and/or adjuvant therapy, a more advanced population, to our knowledge, than the patients typically enrolled in other neoadjuvant trials in stage III melanoma.

METHODS

Trial Design

PIVOTAL is an open-label, randomized, controlled, multicentre phase 3 trial (see **Supplementary Figure 1** for a visual representation of the study design). The trial was conducted at 22 sites in four countries (Germany, Italy, France and Poland) from July 2016 to August 2023. Adult patients with locally advanced malignant melanoma of the skin who had at least one skin or nodal lesion eligible for complete surgical resection were enrolled. Prior antitumor treatment, including surgery, radiotherapy, and approved systemic therapy was allowed.

Patients were randomly assigned in a 1:1 ratio to receive either neoadjuvant daromun followed by surgery (arm 1) or upfront surgery (arm 2). In the experimental arm 1, daromun - a mixture of 13 million IU of L19IL2 and 400 µg of L19TNF - was administered intratumorally to all injectable skin and nodal tumors of the patients, once a week (q1W) for up to 4 weeks followed by surgery within 4 weeks after last treatment.

Patients randomized to the control arm 2 underwent surgical resection of all existing melanoma tumor lesions within 4 weeks from randomization.

Postoperative treatment with EMA-approved adjuvant therapies was allowed in both arms at the discretion of the treating physician (**Supplementary Figure 1** - Study Design).

Patients were observed for disease recurrence until the first recurrence (or unacceptable toxic effects, withdrawal of consent, or death, whichever occurred first) was documented or up to 36 months after randomization. Thereafter, patients were monitored for survival for up to 60 months after randomization.

Data on adverse events (AEs) were collected throughout the trial until 90 days after completion or discontinuation of treatment and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

Comprehensive data regarding clinical, laboratory, and imaging tumor assessments is included in the supplementary materials.

Patient Population

The study's participants were adults (aged ≥18 years) with locally advanced malignant melanoma of the skin (defined as stage IIIB and IIIC according to the 7th edition of the American Joint Committee on Cancer [AJCC] staging system). Patients had to have measurable disease and be candidates for intralesional therapy with at least one injectable skin or nodal melanoma lesion (≥ 10 mm in longest diameter) or multiple injectable lesions with a combined longest diameter ≥ 10 mm. Prior anti-tumor treatments, including surgery, radiotherapy, and approved systemic treatments (e.g., adjuvant immune checkpoint inhibitors, BRAF/MEK inhibitors, etc.) were allowed. An Eastern Cooperative

Oncology Group (ECOG) performance status ≤ 1 and a life expectancy of at least 24 months were prerequisites for enrollment.. The range of acceptable laboratory values at screening was as follows: absolute neutrophil count > 1.5 x 10^9/L, haemoglobin > 9.0 g/dL, platelets > 100 x 10^9/L, total bilirubin ≤ 30 µmol/L (or ≤ 2.0 mg/dl), ALT and AST ≤ 2.5 x the upper limit of normal (ULN), serum creatinine < 1.5 x ULN and a serum LDH level ≤ 1.5 x ULN.

Patients with a diagnosis of uveal or mucosal melanoma, melanoma of unknown primary, or the presence of distant metastases at the time of screening were excluded from the study. The following criteria were used to determine exclusion from the study: the presence of other previous or concurrent cancers (with the exception of cervical carcinoma in situ, treated basal cell carcinoma, superficial bladder tumors, second primary melanoma in situ or any cancer curatively treated ≥ 5 years prior to study entry), the presence of active infections (e.g. requiring antimicrobial therapy), cardiac disease or abnormalities, uncontrolled hypertension, ischaemic peripheral vascular disease (grade IIb-IV), severe diabetic retinopathy, history of organ allograft or stem cell transplantation and autoimmune disease. A comprehensive list of inclusion and exclusion criteria can be found in the protocol and summary of amendments (**Appendix 1**).

Study Assessments

All patients underwent a screening visit within 2 weeks prior to randomization and were required to undergo FDG-PET/CT imaging at the screening visit. Confirmatory biopsies of clinically diagnosed regional melanoma metastases were not mandatory.

Patients randomized to the experimental arm 1 underwent a tumor assessment at the scheduled end of daromun treatment and prior to surgery (within week 5-8 of starting neoadjuvant treatment). Digital photography was used to document the evolution of cutaneous injected lesions at the conclusion of the treatment period. Histopathology was assessed at the treating site on all excised lesions. A review of institutional pathology reports on surgical specimens from patients in arm 1 was performed to assess the rate of pathological complete response. Furthermore, a subset of patients underwent immunohistochemical analysis of tumor-infiltrating lymphocyte populations.

Assessment of human anti-fusion protein antibody (HAFA) formation against L19IL2 and L19TNF was performed in arm 1 patients prior to the first and second dose, on the day of the safety visit and at the first follow-up visit (**Appendices 1 and 2**).

For both arms, tumor assessments were then performed every 3 months after randomization (6 months for imaging by FDG-PET/CT imaging) for a maximum of 36 months after randomization or until evidence of disease progression or until the withdrawal of consent. At each tumor assessment visit, a complete physical examination and regional lymph node sonography were performed. Digital photography was used to document the appearance of local cutaneous recurrences. FDG-PET/CT

scans were routinely recorded every six months or whenever, in the opinion of the treating physician, the occurrence of new visceral lesions was suspected. The recurrence of these lesions was subsequently onfirmed by retrospective Blinded Independent Central Review (BICR) of the imaging data. Furthermore, follow-up visits or telephone contacts (for patients with disease progression) were conducted at least every 6 months up to 60 months to ascertain overall survival.

Adverse event data were collected throughout the study until 90 days after completion or discontinuation of treatment and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.

A comprehensive array of hematological analyses were conducted, encompassing platelet count, red blood cell count, white blood cell count, haemoglobin, and haematocrit. In addition, chemical parameters such as creatinine, blood urea nitrogen, creatine kinase, potassium, sodium, uric acid, chloride, total protein, albumin, LDH, calcium, glucose, phosphorus, and magnesium were measured. Liver function tests (aspartate and alanine aminotransferase, total bilirubin, gamma glutamyl transferase and alkaline phosphatase), coagulation (prothrombin time, activated partial thromboplastin time and INR) were performed for both arms during screening within 2 weeks prior to randomization and at the time of surgery. For arm 1, these analyses were also performed at each treatment visit and on the day of the safety assessment (at one week after the last daromun dose). Pregnancy tests (by serum, when appropriate) were performed at screening in both arms and repeated at the safety visit in arm 1 only.

Primary and Secondary Endpoints

The primary endpoint was recurrence-free survival (RFS) as assessed by BICR and defined as the time from the date of randomization to the date of the first documented disease recurrence or death from any cause after complete surgical resection of the tumor. Patients who did not undergo surgery or were found to have non-completely resectable disease at surgery were censored at the date of the last assessment.

Two interim analyses of RFS were planned at approximately 24 events (25% information fraction), and at 48 events (50% information fraction). The Lan-DeMets alpha spending function was implemented to determine the required nominal alpha level given the information fraction at the time of the analysis.

Secondary endpoints encompassed overall survival (OS), distant metastasis-free survival (DMFS), and pathological response. OS was defined as the time from randomization to death from any cause: alive subjects were censored at the date of last assessment. DMSF was defined as the time from randomization to first distant metastasis or death without recurrence, whichever came first. Subjects alive and without distant metastasis were censored at the date of last assessment. This endpoint may

be affected by informative censoring as patients with first local recurrence were not followed up for distant metastases.

Histopathological analysis of tumor response to neoadjuvant treatment was performed on all surgically removed lesions. Given the absence of specific guidance for assessing the pathological response and the lack of a centralized pathology review, a review of institutional pathology reports on surgical specimens from arm 1 patients was performed to assess the rate of pathological complete response.

Full details and a full list of secondary and exploratory endpoints can be found in the protocol and summary of amendments (**Appendices 1 and 2**).

Trial oversight

The study was funded by Philogen S.p.A. The original protocol and all amendments were approved by the institutional review board or independent ethics committee at each participating site and by the relevant national authorities. Written informed consent was obtained from study participants and approved by the institutional review board at each site. The trial was conducted in accordance with ethical guidelines including Good Clinical Practice standards and the principles of the Declaration of Helsinki.

The study design was reviewed by external scientific experts and by personnel of the sponsor (Philogen S.p.A.). The sponsor funded the study, maintained the study database, and was involved in data collection, data analysis, data interpretation, and the writing of this report. The data were analysed and interpreted by the authors, who wrote the article without any additional writing assistance.

All the authors had access to the full data used in the manuscript and vouch for the accuracy and completeness of the data and for the trial's adherence to the protocol.

Statistical analysis

The primary endpoint, recurrence-free survival (RFS), defined as the time from the date of randomization to the date of the documented first disease recurrence or death from any cause after complete surgical resection of the tumor, was assessed in the intention-to-treat population (ITT), which included all randomized patients.

In order to rule out a potential systemic bias, i.e., censoring imbalance between study arms, a simple reverse K-M analysis was performed. To assess the censoring rates of the survival curves with the reverse K-M approach, the status of the time-dependent outcome for individual patients is flipped: censoring is treated as the ‘event’ (i.e., pseudo-event) of interest and the original event as ‘censored’

(i.e., pseudo-censoring). [13] The resulting reversed K-M plot represents the cumulative probability of individuals being censored at a certain follow-up time. In addition, this method allows the use of the Cox proportional hazards modeling to compare the censoring rates between study arms.

RFS curves were estimated using the Kaplan Meier method and compared using the log-rank test. Ninety-five events would provide an 85% power with a two-sided alpha level of 5% to demonstrate superiority of the neoadjuvant arm for the final analysis, assuming a median RFS of 19.3 months in the neoadjuvant group and 10.4 months in the surgery only group.

OS was a key secondary objective. The median OS time was assumed to be 40 and 72 months for the control and the neoadjuvant arms, respectively, giving a hazard ratio of 0.557. For a log-rank test to compare Kaplan-Meier curves for OS with a two-sided alpha level of 0.05 and 85% of power, 104 death events were required, giving a total sample size of 214 (107+107) subjects, assuming a censoring rate of 10%.

Sample size calculations for this study design were performed using R 3.6.1 (package gsDesign version 3.0-1).

Full details of the planned statistical analysis can be found in the supplementary materials.

RESULTS

Patients

Three hundred fifty-four patients were screened between June 2016 and May 2023: 246 patients were randomized to one of the study arms and included in the ITT analysis (122 in arm 1, and 124 in arm 2). The reasons for screen failures are detailed in the Consort Diagram **(Supplementary Figure 2 - CONSORT Diagram)**. At the database cut-off date (3 May 2023), 5 patients in arm 1 and 3 patients in arm 2 had not yet undergone surgery; 13 patients in arm 1 (3 disease progression; 3 AEs; 3 consent withdrawal; 2 ineligible; 2 refusal of further treatment) and 5 patients in arm 2 (4 consent withdrawal; 1 disease progression) discontinued before surgery (**Supplementary Figure 2 - CONSORT Diagram**).

The demographic and clinical characteristics of the patients were well balanced between the two arms with respect to age and sex distribution, stage of disease at study entry, number and size of lesions, ECOG performance status, and LDH levels (**Table 1**). At screening, 55.3% of patients had skin metastases, 52.4% lymph node metastases, and 0.8% soft tissue metastases:107 patients (43.5%) had ≥2 lesions. 182 (74%) had undergone ≥2 prior surgical procedures for prior recurrence and 86 (35%) had received prior systemic therapy, mainly immune checkpoint inhibitors (ICI) (**Table 1**).

At the database lock date of 3 May 2023, a total of 130 of the 246 randomised participants were censored for administrative (64, 26.4%) or non-administrative (66, 26.8%) reasons in the primary efficacy analysis of RFS. A sensitivity reverse Kaplan-Meier analysis was performed in which the 57 and 73 censored patients in the Arm 2 and Arm 1, respectively, were considered to have an event at the time of their last observation, while the 116 patients were censored at the time of their last observation or event. The resulting plot of reverse Kaplan-Meier curves from randomization for all study participants by arm is shown in **Supplementary Figure 3**. The timing and proportion of censoring was comparable between the two study arms. Using a Cox model with treatment group as the only covariate, the hazard ratio with 95% confidence interval is 1.00 [0.70, 1.43].

Efficacy

Two interim analyses were performed at 25% and 50% of the expected RFS events. An independent Data Safety Monitoring Board (DSMB) concluded that the criteria for futility were not met and recommended that the study continue as planned. The final RFS analysis was performed when the pre-planned 95 events, as adjudicated by the local investigators, were

reported. However, a Blinded Independent Central Review (BICR) identified 20 more events (8 in arm 1 and 12 in arm 2) than the Investigators at the database cut-off date.

At a median follow-up of 22.2 months for arm 1 and 20.2 months for arm 2, 49 events (32 distant and 17 local recurrences) were observed in the daromun arm and 66 (37 distant and 26 local recurrences, and 3 non-treatment-related deaths) in the control arm.

Recurrence-free survival was significantly longer in the neoadjuvant arm than in the upfront surgery arm **(Figure 1**; median RFS: 16.7 months vs 6.8 months; estimated 2-year RFS: 41.6% (95% CI 31.4-55.1%) vs 23.6% (95% CI 15.4-36.2%); HR=0.59 [95% CI 0.41-0.86]; log-rank p=0.005). The investigator-assessed RFS was consistent with the primary analysis by the BICR. Median recurrence-free survival was 24.2 months in the experimental arm and 10.7 months in the control arm, with a 39% reduction in the risk of recurrence or death in patients treated with daromun (HR=0.61 [0.41-0.92]; log-rank p=0.018) **(Supplementary Figure 4**).

Because of the difference in the time between randomization and surgery in the two arms (median time to surgery: 6.6 weeks in arm 1 and 2.3 weeks in arm 2), a time-dependent bias in the assessment of events cannot be ruled out. In a sensitivity landmark analysis at 3 months post randomization, when 99% of surgery had been performed in both arms, the estimated RFS at 2 years was 37.9% (95% confidence interval [CI], 27.4% to 52.3%) in the neoadjuvant-treated group and 24.0% (95% CI, 15.2% to 37.8%) in the surgery only group (HR: 0.60 [0.41-0.89]) (**Supplementary Figure 5**).

The use of post-operative adjuvant therapies was allowed at the discretion of the treating physician in both arms: 31 patients in arm 1 and 47 in arm 2 received postoperative adjuvant therapy (**Supplementary Table 1**). In a planned sensitivity analysis, patients who had been treated with neoadjuvant daromun appeared to have a longer RFS than patients without neoadjuvant treatment both in the patients with post-operative adjuvant therapy (estimated 2-year RFS: 47.2% in arm 1 vs 35.2% in arm 2), and in the patients without post-operative treatment (estimated 2-year RFS: 31.7% in arm 1 vs 13.3% in arm 2) (**Supplementary Table 2**).

Subgroup analysis was performed to evaluate the effect of baseline characteristics such as age, gender, stage, number of lesions, BRAF V600 mutation status and prior treatment on the primary endpoint. The benefit of neoadjuvant daromun was consistent across all patient subgroups (**Figure 2).**

The benefit of neoadjuvant daromun was observed also in distant metastasis recurrence-free survival (DMFS). The estimated DMFS at two years was 59.4% (95% confidence interval [CI], 48.0% to 73.6%) in the neoadjuvant-treated group and 38.8% (95% CI, 26.9% to 56.0 %) in

the surgery alone group (**Figure 3**). The median time to distant metastases was 28 months [95% CI 22.1-NR] in the daromun arm compared to 17.7 months [95% CI 11.2-30.1] in the control arm (HR=0.60; 95% CI [0.37; 0.95]; p=0.029). In accordance with the protocol, data were obtained only for the first disease recurrence, whether local or distant.

OS was another secondary endpoint: 40 deaths had been reported by the database cut-off date. This small number of deaths precluded a definitive comparison between the groups with respect to OS.

A review of the institutional pathology reports confirmed a complete pathological response (defined as no viable tumor in the surgical specimen including skin and nodal lesions) in 22 of 104 patients (21%) who had undergone surgery after neoadjuvant therapy.

Safety

AEs of any cause of grade ≥ 3 were reported in 37 patients (31.3%) in the neoadjuvant arm and in 13 patients (10.9%) in the control arm (**Table 2**). Grade 3 treatment related AEs (TRAEs) were reported in 26.3% of patients in the daromun arm with only one grade 4 (neutropenia) (0.8%), while in the control arm grade 3 TRAEs were reported in 6.7% of patients without any grade 4 (Error! Reference source not found.). The most commonly reported grade 3 TRAE in patients treated with daromun was injection site reaction (11% of patients). Three irAEs were reported in the neoadjuvant arm, which were grade 1 and transient, and there were no treatment-related deaths.

DISCUSSION

Neoadjuvant and perioperative immunotherapies are gaining ground in clinical practice for resectable stage III melanoma, as reflected in the latest ESMO and ASCO guidelines [3,4,14,15]. Data from prospective randomized trials have demonstrated a significant event-free survival (EFS) benefit for immune checkpoint inhibitors in treatment-naïve patients with resectable disease [1,2] whereas OS data in these studies were immature at the time of reporting. Intralesional neoadjuvant immunotherapy with T-VEC was also evaluated in a randomized phase 2 trial:[5] sustained, although not statistically significant, improvements in 5-year RFS and OS were reported with neoadjuvant T-VEC plus surgery compared to standard surgery.

PIVOTAL is the first phase 3 trial of intralesional neoadjuvant targeted immunocytokines, daromun, followed by surgery versus upfront surgery in patients with resectable clinical stage III melanoma, many of whom had experienced multiple recurrences (74% with ≥2 prior surgeries) and had received prior systemic treatments (35%). The population of patients in the PIVOTAL trial is different from that in the SWOG 1801 or NADINA trials. In fact, in the latter studies, prior immunotherapy with ICIs was an exclusion criterion, whereas in the SWOG-1801 only 4 out of 313 patients (1.3%) had received prior targeted therapy with BRAF/MEK inhibitors.[1,2] In the T-VEC phase II study only 4% of the patients had received prior systemic immunotherapy.[5] Finally, 40.6% of patients in PIVOTAL had in-transit metastases, which have adverse prognostic implications,[12] while 10.4% of patients in NADINA had in-transit metastases.

The prognostic significance of the study population is underscored by the strikingly short median RFS observed in the control arm - 5.3 months in patients who received no postoperative adjuvant therapy and 11.7 months in those who received postoperative adjuvant therapy. Both median RFS are significantly shorter than RFS benchmarks in recent adjuvant trials (e.g., COMBI-AD >2.8 years, [16]; KEYNOTE-054 >15 months [17]). This fact underlines that PIVOTAL investigated a high-risk population that was not represented in previous neoadjuvant trials, which enrolled relatively homogeneous, treatment-naïve populations and excluded patients with prior systemic therapy.

Despite the more advanced patient population, PIVOTAL showed a significant improvement in RFS with a 41% reduction in the risk of recurrence or death in patients treated with daromun. In the pre-specified analysis of DMFS, daromun significantly reduced the risk of distant

metastasis by 40% compared to the control arm. These findings suggest that daromun triggers a systemic immune response, even when administered locally.

The open-label design of PIVOTAL and the lack of risk factor stratification may be of potential concern. Although the long enrollment period and the lack of stratification criteria may have increased the likelihood of heterogeneity between the two arms, there is no imbalance between the two arms in terms of baseline patient characteristics. In addition, the forest plot in Figure 2 shows that none of the factors considered appear to affect the activity of intralesional daromun. The results of an additional analysis regarding the distribution of patients with/without pre-study systemic treatments and with/without post-operative adjuvant treatment between the two arms are detailed in the Supplementary Materials (**Supplementary Table 3** and **Supplementary Figure 6**).

The longer time from randomization to surgery in the neoadjuvant arm compared to the control arm, may have introduced a time-dependent bias in the observation of events for the primary efficacy analysis. A post-hoc landmark analysis accounting for the longer time-to-surgery in the neoadjuvant arm still shows a >50% increase in 2-year RFS in the neoadjuvant group compared to the upfront surgery group.

Patients could not be stratified by planned post-operative adjuvant therapy: adjuvant ICI was only allowed at the treating physician's discretion after its approval in Europe in mid-2018. In a sensitivity analysis, post-hoc stratification was performed and, when RFS for patients with or without post-operative adjuvant systemic therapy was analysed using Kaplan-Meyer curves, the prolongation of RFS with neoadjuvant Daromun appeared to be maintained both in patients receiving adjuvant ICI and in those without post-operative treatment.

Patients who receive neoadjuvant therapy are at risk of losing the opportunity for curative surgery due to disease progression or treatment-related SAEs prior to surgery. Evidence to date suggests that approximately 5% of patients enrolled in neoadjuvant trials with stage III disease at initial assessment will progress to distant metastatic disease before surgery. [18, 19] Only 4.9% of patients in the neoadjuvant daromun arm experienced disease progression (3 patients) or AEs (3 patients) leading to inability to undergo surgery.

From a safety standpoint, daromun was well tolerated, with most adverse events limited to local injection site reactions. Immune-related AEs were infrequent (2.5%) and mild. In contrast to systemic neoadjuvant regimens, which have reported Grade ≥3 AEs in up to 47% of patients (e.g., NADINA), daromun demonstrated a favorable safety profile with significantly lower rates of high-grade toxicity (28.8%) and serious AEs (10.2%).

Neoadjuvant daromun represents a well-tolerated and effective therapeutic option for patients with pretreated, recurrent stage III melanoma. Its favorable safety profile and clinical efficacy support its potential role as a complementary or alternative strategy to systemic immunotherapy, particularly in high-risk patients or those ineligible for immune checkpoint inhibitors.

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LEGENDS TO TABLES

Table 1: Baseline demographic and clinical characteristics for the ITT population at the time of randomization.

The data shown are for the intention-to-treat population, which included all patients who underwent randomization between the two treatment arms (daromun + surgery and surgery without neoadjuvant therapy). A chi-square test indicated that there was no statistically significant difference in the distribution of baseline characteristics between the two arms of the study.

Table 2: Adverse events in the safety-evaluable population.

The safety population included all patients who were randomly assigned to any of the two study arms and who had received at least one dose of daromun and/or underwent surgery (arm 1), or who underwent surgery (arm 2). Data are n (%) of patients who experienced adverse events.

*Safety population of both arms included also patients who were scheduled for surgery at the database cutoff date: 5 patients in arm 1 and 3 patients in arm 2.

**In a retrospective analysis, 3 patients treated with ≥ 1 dose of daromun (2.5%) experienced an episode of immune-related Adverse Events (irAEs) characterized by mild (CTCAE G1) severity: immune-mediated thyroiditis, dermatitis and maculo-papular rash.

LEGENDS TO FIGURES

Figure 1: Kaplan-Meier estimates of recurrence-free survival in the ITT population.

Shown are estimates of recurrence-free survival for 246 patients in the study (ITT population) stratified by treatment group. The results were assessed by retrospective blinded independent central review (BICR). Tick marks show data censored at the time of last disease assessment. The hazard ratio (HR) in the table results from a univariate Cox regression with the treatment arm as the covariate. The proportional hazards assumption was verified using Schoenfeld residuals.

Figure 2: Forest Plot of recurrence-free survival across subgroups according to baseline characteristics.

Recurrence-free survival in prespecified subgroups in the intention-to-treat population for daromun plus surgery (arm 1) versus surgery (arm 2). For each group, HR and 95% CI are estimated using a univariate Cox regression model with assigned arm as a covariate. The x-axis is on a logarithmic scale and the size of the squares is proportional to the number of events in each group.

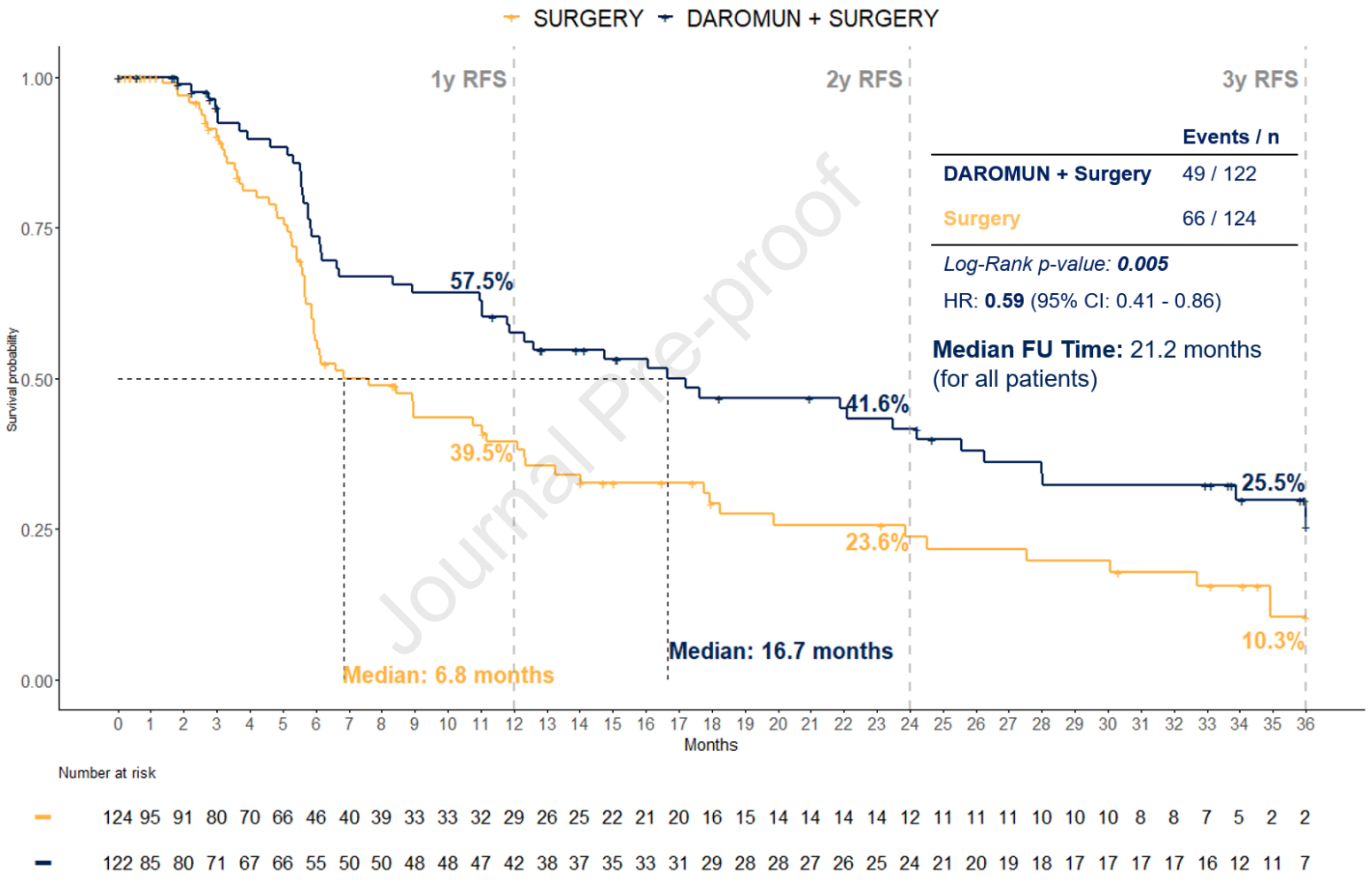
Figure 3: Kaplan-Meier estimates of distant metastasis-free survival in the ITT population.

Kaplan-Meier estimates of distant metastasis-free survival were assessed by retrospective blinded independent central review (BICR). Tick marks show data censored at the time of last disease assessment. DMFS is defined as the time from randomization to distant metastasis without prior local metastasis or death from any cause without recurrence.

	Daromun + SURGERY (n=122)	SURGERY (n=124)	TOTAL (n=246)
Age — n (%)			
< 65 yr	60 (49.2)	65 (52.4)	125 (50.8)
≥ 65 yr	62 (50.2)	59 (47.6)	121 (49.9)
Median age (min - max) — yr	65.0 (23.0 - 88.0)	63.0 (22.0 - 91.0)	64.0 (22.0 - 91.0)
Gender — n (%)			
Female	53 (43.4)	55 (44.4)	108 (43.9)
Male	69 (56.6)	69 (55.7)	138 (56.1)
ECOG — n (%)			
0	113 (92.6)	120 (96.8)	233 (94.7)
1	9 (7.4)	4 (3.3)	13 (5.3)
LDH level — n (%)			
Normal	104 (85.3)	102 (82.3)	206 (83.7)
Elevated	18 (14.8)	22 (17.7)	40 (16.3)
Disease stage — n (%) [AJCC 7th version]			
IIIB	33 (27.0)	33 (26.6)	66 (26.8)
IIIC	60 (49.2)	67 (54.0)	127 (51.6)
III unspecified	24 (19.7)	23 (18.6)	47 (19.1)
IV	5 (4.1)	1 (0.8)	6 (2.4)
Disease stage — n (%) [AJCC 8th version]			
IIIB	23 (18.9)	26 (21.0)	49 (19.9)
IIIC	84 (68.8)	88 (71.0)	172 (69.9)
IIID	10 (8.2)	5 (4.0)	15 (6.1)
III unspecified	-	4 (3.2)	4 (1.6)
IV	5 (4.1)	1 (0.8)	6 (2.4)
BRAF mutation status — n (%)			
Mutated	48 (39.3)	40 (32.3)	88 (35.8)
Wild-type	41 (33.6)	51 (41.1)	92 (37.4)
Unknown	33 (27.1)	33 (26.6)	66 (26.8)
Number of melanoma metastases at study entry			
1	65 (53.3)	74 (59.7)	139 (56.5)
2	26 (21.3)	22 (17.7)	48 (19.5)
≥ 3	31 (25.4)	28 (22.6)	59 (24.0)
Location of melanoma metastasis— n (%)*			
Skin (Cutaneous and Subcutaneous)	72 (59.0)	64 (51.6)	136 (55.3)
Lymph nodes	64 (52.5)	65 (52.4)	129 (52.4)
Soft Tissue	1 (0.8)	1 (0.8)	2 (0.8)
Other	2 (1.6)	1 (0.8)	3 (1.2)
Prior surgery — n (%)			
None	11 (9.0)	9 (7.3)	20 (8.1)
1	18 (14.8)	26 (21.0)	44 (17.9)
2	44 (36.1)	41 (33.1)	85 (34.6)
≥ 3	49 (40.2)	48 (38.7)	97 (39.4)
Prior radiotherapy — n (%)			
No	115 (94.3)	120 (96.8)	235 (95.5)
Yes	7 (5.7)	4 (3.2)	11 (4.5)
Prior systemic therapy — n (%)			
No	79 (64.8)	81 (65.3)	160 (65.0)
Yes	43 (35.3)	43 (34.7)	86 (35.0)
Immunotherapy only	36 (83.7)$	30 (69.8)$	66 (76.7)
Targeted Therapy only	4 (9.3)	5 (11.6)	9 (10.5)
Immunotherapy and Targeted Therapy	1 (2.3)	3 (7.0)	4 (4.7)
Immunotherapy and Chemotherapy	1 (2.3)	2 (4.7)	3 (3.5)
Chemotherapy only	-	2 (4.7)	2 (2.3)
Immunotherapy and Clinical Trial	1 (2.3)**	1 (2.3)***	2 (2.3)

Data are n (%). Percentages might not total 100 because of rounding.
* Patient can have multiple lesions (double counting)
$ Prior ICI – n (%): daromun + surgery 21 (17.2); surgery 19 (15.3)
** Pembrolizumab versus placebo
*** SGI-110 (DNMT inhibitor)

Adverse Event, n (%)	Daromun + Surgery (n=118)*			Surgery (n=119)*		
	ANY GR	GR = 3	GR = 4	ANY GR	GR = 3	GR = 4
Any AEs	113 (95.8)	34 (28.8)	3 (2.5)	58 (48.7)	13 (10.9)	-
Any SAEs	18 (15.3)	12 (10.2)	2 (1.7)	10 (8.4)	8 (6.7)	-
Any TRAEs	110 (93.2)	31 (26.3)	1 (0.8)	40 (33.6)	8 (6.7)	-
Any TRSAEs	10 (8.5)	8 (6.8)	-	5 (4.2)	4 (3.4)	-
irAEs**	3 (2.5)	-	-	0	-	-
TRAEs in >10% of patients, n (%)						
Injection site reaction	79 (66.9)	13 (11.0)	-	0	-	-
Pyrexia	57 (48.3)	1 (0.8)	-	0	-	-
Chills	57 (48.3)	-	-	0	-	-
Nausea	26 (22.0)	-	-	0	-	-
Fatigue	18 (15.3)	1 (0.8)	-	0	-	-
Headache	16 (13.6)	-	-	0	-	-
Influenza like illness	14 (11.9)	-	-	0	-	-
Vomiting	14 (11.9)	-	-	0	-	-
Alanine aminotransferase increased	12 (10.2)	1 (0.8)	-	0	-	-



Subgroup	DAROMUN + Surgery	Surgery	HR (95% CI)
All patients	49/122	66/124	0.6 (0.4 to 0.9)
Age			
< 65	23/60	34/65	0.5 (0.3 to 0.9)
≥ 65	26/62	32/59	0.7 (0.4 to 1.1)
Gender			
Male	33/69	37/69	0.7 (0.4 to 1.1)
Female	16/53	29/55	0.5 (0.3 to 0.9)
ECOG - PS			
0	44/113	65/120	0.6 (0.4 to 0.9)
1	5/9	1/4	N/A
LDH level			
Normal	43/104	53/102	0.6 (0.4 to 0.9)
Elevated	6/18	13/22	0.7 (0.3 to 1.9)
Disease stage - AJCC 7th			
III B	11/33	16/33	0.4 (0.2 to 0.8)
III C	26/60	38/67	0.6 (0.4 to 1.0)
III unspecified	11/24	12/23	0.9 (0.4 to 2.2)
IV	1/5	0/1	N/A
BRAF mutation status			
Mutated	18/48	16/40	0.8 (0.4 to 1.6)
Wild Type	17/41	31/51	0.4 (0.2 to 0.8)
Unknown	14/33	19/33	0.7 (0.3 to 1.4)
Lesions (n.)			
1	29/65	34/74	0.7 (0.4 to 1.1)
2	9/26	16/22	0.6 (0.2 to 1.3)
≥ 3	11/31	16/28	0.4 (0.2 to 0.9)
Prior Surgery			
None	4/11	4/9	0.7 (0.2 to 2.7)
1	5/18	18/26	0.3 (0.1 to 1.0)
2	18/44	19/41	0.7 (0.3 to 1.3)
≥ 3	22/49	25/48	0.6 (0.3 to 1.0)
Prior RT			
No	45/115	62/120	0.6 (0.4 to 0.9)
Yes	4/7	4/4	0.6 (0.1 to 2.5)
Prior systemic therapy			
No	30/79	43/81	0.6 (0.4 to 1.0)
Yes	19/43	23/43	0.5 (0.3 to 1.0)