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Effect of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF, Sargramostim) Administration on Serum S100B and Its Subtypes in Melanoma Patients

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Updated Abstract

Introduction: Serum S100B seems to be a good tumor marker for hematogenous dissemination of malignant melanoma. However, S100B is also a good histological marker of dendritic cells (DC). In a previous study we found that DC only expressed the S100BB subtype, whereas melanoma expressed the S100A1B and BB subtypes. As GM-CSF can induce activation/expansion of DC, we studied if such therapy also increased serum S100B and analyzed the subtype in patients showing increased S100B.

Patients and Methods: In 43 patients with malignant melanoma stage II (T4), III, and IV, receiving GM-CSF therapy (14 days on and 14 days off for 1 year) after surgical cytoreduction, serum S100B was measured before therapy (d0), following 14 days of subcutaneous GM-CSF (d15), and 14 days off GM-CSF (d29), as well as d155 if possible (n = 30). If serum S100B was increased, subtype analysis was performed with immunoassays for S100A1B and S100BB.

Results: In 40 patients with a normal serum S100B at d0, a significant rise of serum S100B occurred at d15, returning to normal at d29 ($P < 0.001$ for d15 versus d0 and d29). Analysis of the increased S100B at d15 showed increased levels of S100BB in 7/13 patients and no increased levels of S100A1B. In 3 patients S100B was increased at d0, which was of the S100A1B subtype in 1 patient who developed rapidly progressive disease. The other 2 had only increased S100BB levels and have developed no evidence of disease (NED) in over 1.5 years of follow-up. In 30 patients with a normal serum S100B at start, an increase was shown at d155 ($P = 0.006$ and $P = 0.015$ compared to d0 and d29 respectively, but not significantly different from d15). Of the 30 patients evaluated on d155, 9 patients had increased S100B. Two patients showed increased levels of S100A1B and S100BB (1 DOD, 1 AWD). Five patients showed only increased levels of S100BB (2 died of disease and 3 remained NED) and 2 patients had no increased dimers (both NED).

Conclusions: A significant increase in serum S100B was found following 14 days of GM-CSF therapy (d15), which returned to normal again after 14 days without any therapy. No patients with an increased S100B on d15 showed increased S100A1B levels, while 7/13 patients had increased S100BB levels, suggestive of an activation or expansion of DC. On d155, increased levels of S100A1B were indicative of active melanoma, but the absence of an increased level of S100A1B did not exclude active melanoma.

Background

Biologic Activity of GM-CSF

- Induces cytotoxicity of melanoma cells following GM-CSF treatment of peripheral blood monocytes in vitro[1]
- Enhances in vivo monocyte-induced cytotoxicity of HT29 colon carcinoma[2] and antibody-coated xenogeneic cells[3]
- Increases proliferation, maturation, and migration of dendritic cells, also known as *antigen-presenting cells*, which induce T-cell immune responses[4]

Clinical Activity of Adjuvant GM-CSF

- Prolongs overall and disease-free survival in patients with malignant melanoma at high risk of recurrence compared with historical controls[5]
  - Adverse events were minor
  - Established dose of 125 μg/m²/d subcutaneously (SC) for 14 days followed by 14 days rest
- Survival benefit confirmed in follow-on interim analysis of 98 patients[6]

Serum S100B

- Tumor marker for malignant melanoma and histologic marker for dendritic cells
- May predict survival duration in melanoma patients[7,8]
  - Prolonged survival noted in patients with normal serum levels of S100B
  - Elevated S100B levels in patients with metastatic growth or progressive disease
- Shown to provide valuable clinical input for predicting patient response and monitoring during chemoimmunotherapy

Study Objectives

Main Objective

- Determine the effect of adjuvant GM-CSF on immunologic parameters and their potential association with clinical outcome in patients with melanoma

Specific Aims

- Describe the effects of adjuvant GM-CSF on immunologic function
- If immunologic changes occur, associate with safety and clinical outcome (time to disease recurrence, time to disseminated disease, and/or survival)

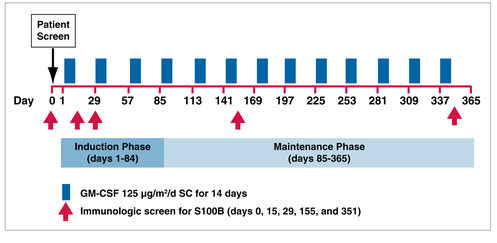
Methods

Study Design

- Pilot study in patients with malignant melanoma who are at high risk of recurrence (stage II [T4], III, and IV) following surgical resection of all known disease
- GM-CSF (sargramostim, Leukine®) 125 μg/m²/d SC for 14 days followed by 14 days without therapy (28-day cycle) for 1 year
  - Clinical status reviewed until patient is tumor-free for 5 years or death
- In event of recurrence, GM-CSF is
  - Continued if tumor is surgically excisable
  - Stopped if patient requires systemic therapy

Treatment Program

Figure 1. Treatment Program for GM-CSF and Immunologic Assessments



Eligibility

Inclusion Criteria

- Age ≥ 14 years
- Histologically proven stage II (T4), III, or IV malignant melanoma that was surgically resected and shows no evidence of disease (NED)
  - Initiation of GM-CSF required within 90 days of surgical resection rendering patient NED
- Completed previous therapy ≥1 month prior to study entry
- Signed Institutional Review Board (IRB)-approved informed consent form

Exclusion Criteria

- Pregnancy or lactating
- If corticosteroid therapy required or receiving other forms of immunotherapy
- If received prior chemotherapy or therapy with GM-CSF

Methods (cont)

Immunologic Testing

- Samples drawn
  - Pretreatment (before treatment)
  - Early induction period (cycle 1)
    - Day 15 (after 14 days on treatment)
    - Day 29 (after 14 days of rest)
  - Maintenance treatment period (cycle 6)
    - Day 155 (after 14 days on treatment)
  - End of treatment period (cycle 13)
    - Day 351 (after 14 days on treatment)
- Serum S100B levels[9]
  - Determined by Elecsys-R-S100B automatic chemoluminescence assay
  - S100A1B and S100BB subtypes determined by CanAg S100 enzyme-linked immunoassay
  - Upper reference values were established as 0.10 μg/L for S100B, and 0.04 μg/L for S100A1B and S100BB

Clinical and Laboratory Evaluations

- Baseline patient history, physical examination, CBC, chemistry panel, CT scan of chest and abdomen (and pelvis if indicated) or PET scan, and MRI or CT scan of brain
- At 14 days prior to cycles starting at 3, 6, and 9 months: interval history, physical examination, blood tests, and scans
  - Less frequent analysis after post-day 85 maintenance
- Patients to keep diary to be monitored at each visit for adverse events and compliance in dosing
- CBC and differential on same schedule as immunologic testing, with additional samples after 14 days of therapy in cycles 3 and 9

Results

Patient Characteristics

Table 1. Baseline Patient Characteristics

Characteristic	Patients (N = 43)
Age (at start of study)	
Median, y ± SEM	57.2 ± 1.8
Range	31.2–83.8
Gender, n (%)	
Male	33 (77)
Female	10 (23)
Stage of disease, n (%)	
IIC	3 (7)
IIIA	5 (12)
IIIB	24 (56)
IIIC	5 (12)
IVM1A	4 (9)
IVM1c	2 (5)

Patient Status

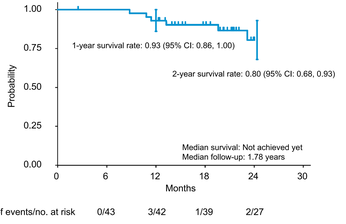
Table 2. Patient Status at Last Follow-Up (N = 43)

Characteristic	Patients (N = 43)
Patient status at last follow-up, n (%)	
Alive	37 (86)
Dead*	6 (14)
Duration of follow-up, y	
Median	1.78
25th, 75th percentiles	1.27, 2.00
Minimum, maximum	0.21, 2.00
Duration of overall survival, y	
Median, 25th and 75th percentiles	Not achieved yet
Minimum, maximum	0.21, 2.00

*All patients died of their disease and not from other causes.

Results (cont)

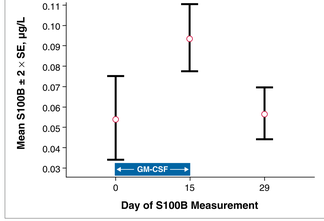
Figure 2. Kaplan-Meier Estimates of 1-Year and 2-Year Survival Rates Measured From the Date of Surgery (N = 43)[10]



Serum S100B

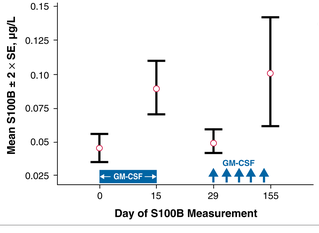
Serum S100B Levels Over the Course of Treatment

Figure 3. Mean Serum S100B Levels at Days 0, 15, and 29 (N = 40)



- 40 patients showed normal S100B levels at day 0
  - S100B was increased in 3 patients at day 0
- Mean serum S100B levels at day 15 are significantly different from those at day 0 or 29 ($P < .001$)

Figure 4. Mean Serum S100B Levels at Days 0, 15, and 29 Compared With Levels on Day 155 (N = 30)



- Mean serum S100B levels in 30 patients at day 155 are significantly different from those at day 0 ($P = .005$) or day 29 ($P = .008$)

Results (cont)

Serum S100B Levels Over the Course of Treatment (cont)

- In 40 patients with a normal serum S100B at day 0 (mean 0.04 ± SD 0.02 μg/L), a significant rise in serum S100B occurred at day 15 (mean 0.09 ± SD 0.04 μg/L), returning to normal at day 29 (mean 0.05 ± SD 0.02, $P < 0.001$ for day 15 versus day 0 and day 29)
- In 30 patients with a normal serum S100B at start, an increase was shown at day 155 compared with day 0 and day 29 ($P = 0.006$ and $P = 0.015$, respectively), but was not significantly different from day 15

Analysis of Serum S100B Subtype Levels

- Day 0
  - S100B was increased in 3 patients
    - The S100A1B subtype was increased in 1 patient who developed rapidly progressive disease during the first month of GM-CSF therapy
    - The S100BB subtype was increased in 2 patients who have not developed evidence of metastatic disease during more than 1.5 years of follow-up
- Day 15
  - Elevated S100BB in 7 of 13 patients
  - No increase in S100A1B

Table 3. Subtype Analyses of Patients With Increased S100B at Day 155

S100B Subtype	No. of Patients (N = 9)	Patient Status
Elevated S100A1B and S100BB	2	1 DOD, 1 AWD
Increased S100BB only	5	2 DOD, 3 NED
No increased dimers	2	NED

AWD = alive with disease, DOD = died of disease, NED = no evidence of disease.

Conclusions

- At a median 1.78 year follow-up, patients showed an estimated 1- and 2-year survival rate of 93% and 80%, respectively
- GM-CSF treatment induced a significant increase in serum S100B levels after 14 days of treatment (day 15)
- Serum S100B levels returned to normal following 14 days without any therapy (day 29)
- Subtype analysis showed that 7 of 13 patients had increased S100BB at day 15, suggesting dendritic cell activation or expansion
- Subtype analysis indicated that no patients with elevated S100B showed increased S100A1B levels on day 15
- In follow-up, increased S100A1B levels appeared indicative of active melanoma, whereas its absence did not exclude metastatic disease

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