The Oncologist®

Clinical Trial Results

Endogenous Heat-Shock Protein Induction with or Without Radiofrequency Ablation or Cryoablation in Patients with Stage IV Melanoma

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Trial Information

- **ClinicalTrials.gov Identifier**: NCT00568763
- **Sponsor(s)**: Mayo Clinic
- **Principal Investigators**: Svetomir N. Markovic, James M. Heun, Evidio Domingo-Musibay
- **IRB Approved**: Yes

Lessons Learned

- Percutaneous thermal ablation combined with in situ granulocyte-macrophage colony-stimulating factor cytokine therapy was technically feasible and well tolerated.
- No significant clinical or immunologic responses were seen.

Abstract

Background. Melanoma tumor-derived heat-shock proteins (HSPs) and HSP-peptide complexes can elicit protective antitumor responses. The granulocyte-macrophage colony-stimulating factor (GM-CSF) chemokine can also promote uptake and processing by professional antigen presenting cells (APCs). On this basis, we designed a pilot study of percutaneous thermal ablation as a means to induce heat-shock protein vaccination plus GM-CSF to determine safety and preliminary antitumor activity of this combination.

Materials and Methods. This study was designed to assess overall safety of percutaneous ablation combined with GM-CSF for unresectable, metastatic melanoma including uveal and mucosal types. All patients received heat-shock therapy (42°C for 30 minutes), then received one of three treatments: (a) intralesional GM-CSF (500 mcg standard dose); (b) radiofrequency ablation (RFA) + GM-CSF; or (c) cryoablation plus GM-CSF. The primary endpoint of the study was the induction of endogenous HSP70 and melanoma-specific cytotoxic T lymphocytes (CTL).

Results. Nine patients (three per study arm) were enrolled. No dose-limiting toxicity was observed as specified per protocol. All patients developed progressive disease and went on to receive alternative therapy. Median overall survival (OS) was 8.2 months (95% confidence interval [CI] 2–17.2). The study was not powered to detect a difference in clinical outcome among treatment groups.

Conclusion. Percutaneous thermal ablation plus GM-CSF was well tolerated, technically feasible, and demonstrated an acceptable adverse event profile comparable to conventional RFA and cryoablation. While HSP70 was induced following therapy, the degree of HSP70 elevation was not associated with clinical outcome or induced CTL responses. While percutaneous thermal ablation plus GM-CSF combinations including checkpoint inhibitors could be considered in future studies, the use of GM-CSF remains experimental and for use in the context of clinical trials. ***The Oncologist*** 2017;22:1026–e93

Discussion

Metastatic melanoma has historically carried poor prognosis [1], but new approaches using combination immunotherapy [2], molecularly targeted agents [3], and radiation therapy [4] are rapidly changing the outlook of the disease. Estimates for 2017 predict approximately 87,110 new cases of melanoma and 9,730 deaths from the disease [5]. Objective response rates (ORR) in previously untreated patients on anti-programmed death receptor-1 (PD-1) therapy approach 40%; still more than half of patients fail to respond [6, 7]. For uveal melanoma patients, response rates are significantly worse, with an ORR of 3.6% and median PFS and OS of 2.6 months and 7.6 months, respectively [8].

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Multimodality and multidisciplinary management of metastatic disease includes the use of percutaneous thermal ablation, a modality associated with durable local control and oncologic outcomes comparable to surgery [9]. In this study, we sought to evaluate whether in situ melanoma vaccination could be achieved by three local directed therapies combined with intralesional GM-CSF. We show that heat-shock therapy, RFA, and cryoablation are all associated with an increase in HSP70 levels following therapy; however, we did not detect significant induction of anti-melanoma T-cell responses, a prespecified endpoint of the study.

Therapeutic strategies that help generate an immunostimulatory tumor microenvironment may help inform clinical approaches to treat patients with refractory disease. Response to current checkpoint inhibitor strategies depends on antitumor T cells expressing PD-1 and correlates with programmed death-ligand 1 (PD-L1) expression on tumor cells. Here we have shown that thermal ablation therapy for melanoma induces expression of HSP70, a melanoma tumor-associated antigen and alarmin molecule with immunological adjuvant activity. Several studies have shown that heat-shock protein complexes containing tumor-derived proteins are released and can be processed by antigen presenting cells [10, 11]. In this study, cryoablation was associated with the most significant plasma HSP70 elevations, although it is not clear that this is necessary or sufficient for effective in situ vaccination. However, local expression of GM-CSF by the modified oncolytic herpes simplex virus talimogene laherparepvec has shown promise [12]. The cellular immunologic responses to viruses, as well as differences in local tumor dose of GM-CSF expressed by oncolytic viruses, may in part explain some of the difference in observed outcomes between the two approaches.

Further refinement of this in situ vaccination therapy strategy is still required prior to further development of the approach. Testing of lower doses of GM-CSF in combination with checkpoint inhibition may prove more fruitful, particularly in patients with uveal melanoma, for whom responses to checkpoint inhibitors are low and the presence of liver-predominant metastatic disease can be successfully targeted for percutaneous ablation therapy.

Trial Information	
Disease	Melanoma
Stage of Disease/Treatment	Metastatic/Advanced
Prior Therapy	No designated number of regimens
Type of Study - 1	Phase I
Type of Study - 2	Null
Primary Endpoint	Tolerability
Primary Endpoint	Correlative endpoint
Secondary Endpoint	Deliverability
Secondary Endpoint	Toxicity
Secondary Endpoint	Efficacy
Additional Details of Endpoints or Study Design	Correlative endpoints: Plasma HSP70 levels and doubling of anti-melanoma CTLs
Investigator's Analysis	Drug tolerable, efficacy indeterminant

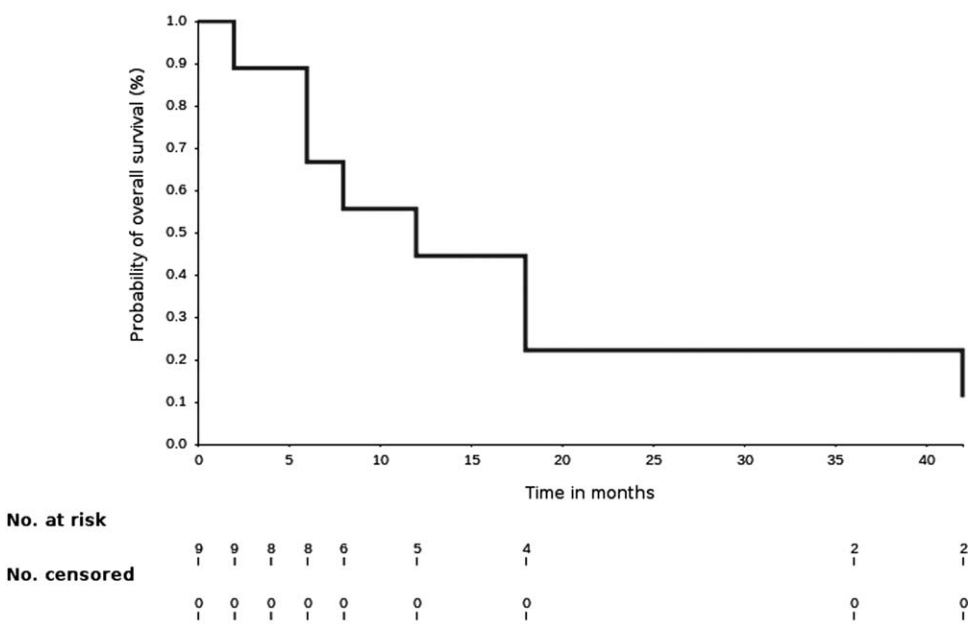
Drug Information for Phase I Thermal Ablation	
Drug 1	
Generic/Working name	Sargramostim/GM-CSF
Trade name	Leukine
Company name	Sanofi
Drug type	Cytokine
Drug class	Immune therapy
Dose	500 Micrograms (mcg) per flat dose
Route	Intra-tumoral
Schedule of administration	Once

Patient Characteristics for Phase I Thermal Ablation	
Number of patients, male	3
Number of patients, female	6
Stage	IV
Age	Median (range): 63 (45–81)
Number of prior systemic therapies	Median (range): 2 (0–4)

Performance Status: ECOG	0 — 9 1 — 2 — 3 — unknown —
Cancer Types or Histologic Subtypes	Mucosal melanoma: 3 Ocular melanoma: 3 Cutaneous melanoma: 2 Acral-lentiginous melanoma: 1

Primary Assessment Method for Phase I Thermal Ablation	
Assessment	
Number of patients screened	13
Number of patients enrolled	11
Number of patients evaluable for toxicity	9
Number of patients evaluated for efficacy	9
Response assessment PD	$n = 9$ (100%)
(Median) duration assessments OS	8.2 months, CI: 2–17.2
Kaplan-Meier time units	Months

Time of scheduled assessment and/or time of event	No. progressed (or deaths)	No. censored	Percent at start of evaluation period	Kaplan-Meier %	No. at next evaluation/ No. at risk
0	0	0	100.00	100.00	9
2	1	0	100.00	88.89	8
4	0	0	88.89	88.89	8
6	2	0	88.89	66.67	6
8	1	0	66.67	55.56	5
12	1	0	55.56	44.44	4
18	2	0	44.44	22.22	2
36	0	0	22.22	22.22	2
42	1	0	22.22	11.11	1



Kaplan-Meier curve for overall survival (OS) of all nine patients enrolled on this study.

Phase I Thermal Ablation Adverse Events

Of Special Interest, All Cycles							
Name	**NC/NA**	**1**	**2**	**3**	**4**	**5**	**All grades**
Bone pain	100%	0%	0%	0%	0%	0%	0%
Tumor pain	67%	0%	22%	11%	0%	0%	33%
Fatigue	78%	22%	0%	0%	0%	0%	22%
Thromboembolic event	78%	0%	0%	11%	11%	0%	22%
Confusion	89%	0%	0%	11%	0%	0%	11%
Anemia	89%	0%	11%	0%	0%	0%	11%
Headache	89%	0%	0%	11%	0%	0%	11%
Peripheral motor neuropathy	89%	0%	0%	11%	0%	0%	11%
Peripheral sensory neuropathy	89%	0%	11%	0%	0%	0%	11%

Abbreviation: NC/NA, no change from baseline/no adverse event.

Assessment, Analysis, and Discussion

Completion	Study completed
Investigator's Assessment	Drug tolerable, efficacy indeterminant

Heat-shock proteins (HSPs), also known as molecular chaperones, form an intracellular network of molecular machinery that maintain the proteome. Mammalian HSPs are classified according to molecular weight into families: HSP110, HSP90, HSP70, HSP60, HSP40, and small HSPs (HSP27 and others). Humans encode at least 13 members of the HSP70 family that use the chemical energy provided by ATP hydrolysis to orchestrate cotranslational folding of nascent polypeptide chains [13, 14].

HSP70 family members can bind apoptotic protease activation factor 1 (APAF-1) and Bax proteins in the cytoplasm and inhibit apoptosome formation and proapoptotic activity, respectively [15]. Client proteins for the HSP70 chaperone include mutated BRAF as well as activated phosphorylated focal adhesion kinase (p-FAK, Y397), associated with melanoma invasiveness and metastasis [16]. Extracellular HSP70 peptide complexes released from tumor cells can also activate anti-melanoma T cells in vitro [11] and, in vivo, are bound by and internalized via scavenger receptors, such as CD91 and LOX-1, on the surface of antigen presenting cells [17–20]. In this way, antigen presenting cells (APCs) such as dendritic cells receive tumor antigens that can be processed and presented on major histocompatibility complex class I and class II molecules to CD8 positive and CD4 positive T cells. This process has been termed HSP vaccination, and there is long-standing interest in the use of HSP peptide complexes in tumor vaccination strategies [21].

In the present study, we combined percutaneous ablation therapy and intralesional granulocyte-macrophage colony-stimulating factor (GM-CSF) to couple the DC-stimulatory activity of GM-CSF with the antigenic stimulation of the ablation procedure. We show that the combination was well tolerated and that ablation therapy increases plasma HSP70 levels, demonstrating effective antigen mobilization by the procedure. We also demonstrate feasibility of using intralesional cytokine therapy for in situ vaccination following ablation therapy. Studies combining HSP70 vaccination with interleukin 2 (IL-2), or IL-7/IL-12/IL-15 show improved T cell cytotoxic and proliferative capacity [22], and use of intralesional cytokine combinations as well as other investigational agents (i.e., CpG oligodeoxynucleotides, TLR-agonists) may increase the potency of the approach [23].

Several study limitations are inherent to the small pilot nature of the study. Although a flat 500 mcg dose of GM-CSF was chosen for purposes of this feasibility study, there is a need to define the optimal dose of GM-CSF for HSP vaccination. Furthermore, design of this trial did not permit an assessment of the tumor microenvironment, and future studies would benefit from analysis of therapy-induced changes in the expression of HSPs and checkpoint ligands in biopsy specimens. The chaperone-based vaccination approach we employed was well tolerated, but it was insufficient to produce clinically relevant immunologic activation. Further rational development of HSP vaccination using percutaneous thermal ablation appears likely to require checkpoint inhibition, which has already shown evidence of synergy in murine metastatic melanoma models [24].

Heat-shock proteins play an important role in melanoma tumorigenesis and HSP peptide vaccines have shown promising immunostimulatory activity in pre-clinical and human clinical trials [25]. Future studies exploring combinations with immune checkpoint inhibitors with/without GM-CSF are warranted and will likely translate into improved outcomes for patients managed with percutaneous ablation therapy for metastatic melanoma.

Acknowledgments

We wish to thank Jacob B. Allred for statistical input, and William J. Charboneau for procedural assistance and expertise.

Disclosures

Matthew Callstrom: Neuwave Medical, Medtronic, Thermedical (C/A), Galil Medical (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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Figures and Tables

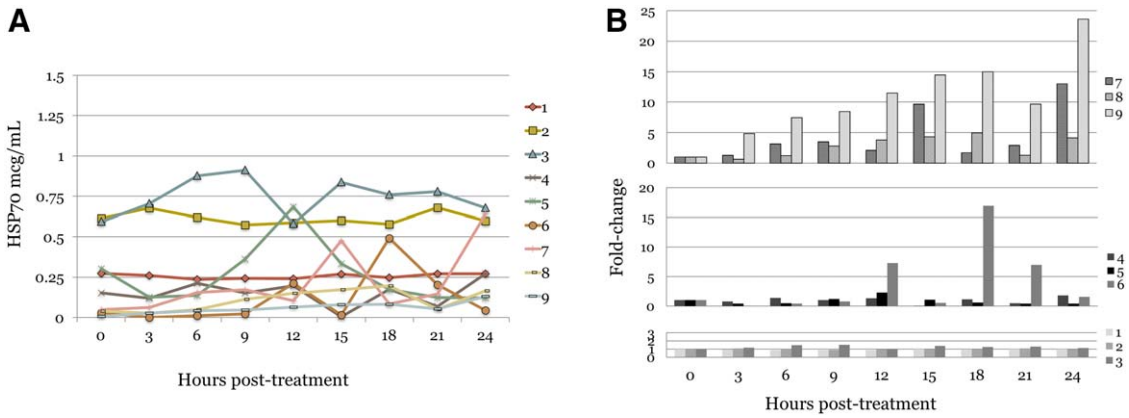

Figure 1. Plasma HSP70 levels. Baseline circulating HSP70 was detectable in all patients. **(A)**: Individual patient levels of plasma HSP70 over 24 hours shown for heat shock therapy (HST), RFA, and cryoablation cohorts. **(B)**: HSP70 fold-change above baseline level measurements for patients receiving cryoablation (top, arm C), RFA (middle, arm B), and HST (lower, arm A), with sustained elevations of HSP70 seen in patients receiving cryoablation therapy.

Abbreviations: HSP70, 70 kilodalton heat-shock protein; RFA, radiofrequency ablation.

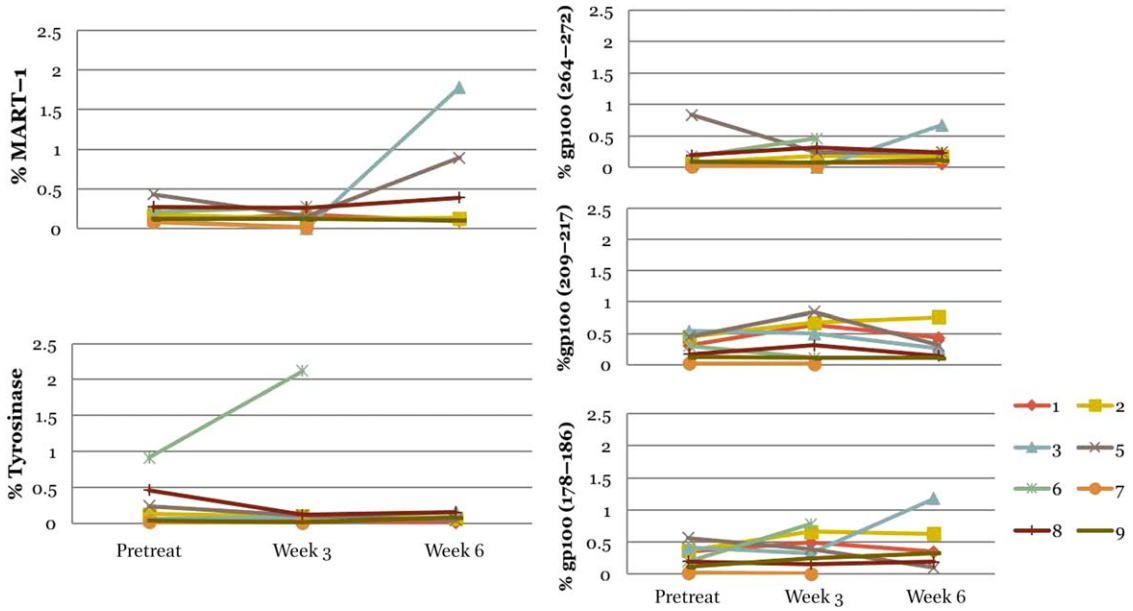

Figure 2. Major histocompatibility complex-tetramer testing identified cluster of differentiation 8 positive (CD8+) cytotoxic T lymphocytes (CTLs) for each tumor antigen. Pre-specified criteria for positive tetramer results was >0.02% tetramer positive CD8+ CTLs if baseline negative, or >2-fold increase in the baseline detectable number. We detected low baseline levels of peripheral CD8+ CTLs against known melanoma antigens MART-1, GP100, and Tyrosinase (7 of 8 evaluable patients). There was no significant induction of CD8+ CTL responses to melanoma antigens following treatment.

Abbreviations: GP100, glycoprotein 100; MART-1, melanoma antigen recognized by T cells 1.