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

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TRIAL INFORMATION
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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) intraleisional 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].
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 intraläsional 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 | Sargramostim/GM-CSF |
|--------|---------------------|
| 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                                | 11.11          | 1                                |
| 42                                              | 1                         | 0            | 22.22                                | 11.11          | 1                                |

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

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RECENT DEVELOPMENTS IN THE INTERSECTION OF MOLECULAR TARGETED THERAPY AND IMMUNE CHECKPOINT INHIBITION

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Table of 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.