function cannot be fully evaluated. Because Treg homing remains intact, we propose that a defect in Treg function (but not necessarily homing) might account for the results observed with the CCR4$^{-/-}$ mice. To test this hypothesis, *in vitro* T-cell suppressor assays may be performed using Tregs isolated from oxazolone-treated skin of WT and CCR4$^{-/-}$ mice.

Additional hypotheses may explain the unexpected results presented by Lehtimäki *et al*. (2010). As discussed previously, multiple cell types, including endothelial cells, DCs, and keratinocytes, produce CCL17. CCR4 may be dispensable with respect to recruitment of T cells into the skin (section 1 in Figure 1), with CCR10 playing a compensatory role in the absence of CCR4, but events downstream of initial recruitment may be important. Secretion of CCL17 by DCs is believed to mediate DC–T-cell interactions. Upon maturation, DCs have been shown to produce CCL17, which mediates attraction and adhesion of these DCs to antigen-primed T cells (Wu *et al.*, 2001). In particular, these interactions may be important for the *in situ* development of effector and regulatory T-cell activation (section 2 in Figure 1). The lack of interaction between DCs and Tregs due to lack of CCR4 on Tregs is another possible explanation for the enhanced inflammation seen in CCR4$^{-/-}$ mice. Finally, keratinocytes also synthesize CCL17, which may be important for epidermotropism of both memory effector T cells and Tregs (section 3 in Figure 1). Correct localization of T-cell populations within the epidermis might alter inflammatory responses. For these reasons, future studies should address events downstream of tissue homing: specifically Treg/DC and T-cell/keratinocyte interactions.

It is becoming clearer that chemokine receptors are not always positive mediators of skin inflammation, but that they can be critical for suppression of inflammation as well. What complicates the picture is that some receptors, as exemplified by CCR4, may act in both ways. Depending on the model of inflammation used, CCR4's effects can influence inflammation in different, almost contradictory, ways. Indeed, the nuances and complexities of chemokine receptor function in the skin are just beginning to be discerned at a fine level.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES
Campbell JJ, Haraldsen G, Pan J *et al*. (1999) The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 400:776–80

Campbell JJ, O'Connell DJ, Wurbel MA (2007) Cutting edge: chemokine receptor CCR4 is necessary for antigen-driven cutaneous accumulation of CD4 T cells under physiological conditions. *J Immunol* 178:3358–62

Duhen T, Geiger R, Jarrossay D *et al.* (2009) Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol* 10:857–63

Fuhlbrigge RC, Kieffer JD, Armerding D *et al.* (1997) Cutaneous lymphocyte antigen is a specialized form of PSGL-1 expressed on skin-homing T cells. *Nature* 389:978–81

Homey B, Alenius H, Muller A *et al.* (2002) CCL27–CCR10 interactions regulate T cell-mediated skin inflammation. *Nat Med* 8:157–65

Hudak S, Hagen M, Liu Y *et al*. (2002) Immune surveillance and effector functions of CCR10(+) skin homing T cells. *J Immunol* 169:1189–96

Lehtimäki S, Tillander S, Puustinen A *et al*. (2010) Absence of CCR4 exacerbates skin inflammation in an oxazolone-induced contact hypersensitivity model. *J Invest Dermatol* 130:2743–51

Lonsdorf AS, Hwang ST, Enk AH (2009) Chemokine receptors in T-cell-mediated diseases of the skin. *J Invest Dermatol* 129:2552–66

Reiss Y, Proudfoot AE, Power CA *et al.* (2001) CC chemokine receptor (CCR)4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. *J Exp Med* 194:1541–7

Sallusto F, Lanzavecchia A, Mackay CR (1998) Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. *Immunol Today* 19:568–74

Sather BD, Treuting P, Perdue N *et al*. (2007) Altering the distribution of Foxp3(+) regulatory T cells results in tissue-specific inflammatory disease. *J Exp Med* 204:1335–47

Wu M, Fang H, Hwang ST (2001) Cutting edge: CCR4 mediates antigen-primed T cell binding to activated dendritic cells. *J Immunol* 167:4791–5

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Paying "Particle" Attention to Novel Melanoma Treatment Strategies

Michele B. Weiss[1] and Andrew E. Aplin[1]

Malignant melanoma remains the deadliest form of skin cancer because of its highly aggressive nature and the lack of effective treatments. Recent investigations into alternative treatment strategies have highlighted the exciting potential of nanoparticles to increase melanoma cell delivery and the efficacy of small interfering RNAs (siRNAs) and pharmacological inhibitors. In this issue, Chen *et al.* report a new liposomal nanoparticle for c-*Myc* siRNA delivery, noting it to be highly effective in reducing c-*Myc* expression and inhibiting melanoma tumor growth in mouse models. This preclinical study underscores the importance of investigating nanoparticle treatment options for chemoresistant melanomas.

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Introduction

The field of targeted therapeutic strategies for melanoma is entering exciting times. Malignant melanoma is the deadliest form of skin cancer and has for decades represented a paradigm for chemoresistance. Current therapeutic options are poor and no new US Food and Drug Administration (FDA)-approved drugs have emerged in recent years. Current melanoma treatment mainstays, such as the alkylating agent dacarbazine and the immune-stimulating agent IL-2, are plagued by a lack of clinical benefit in the majority of patients and numerous side effects. However, highly

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Clinical Implications

- Malignant melanoma is a paradigm for resistance to chemotherapy.
- Nanoparticle delivery of siRNAs represents a selective approach to target any expressed mRNA in melanoma cells.
- c-*Myc* expression is elevated in melanoma, and targeting c-*Myc* shows promise in *in vivo* preclinical assays.

encouraging results have been reported recently, renewing hope that new FDA-approved drugs will be forthcoming. The phase I trial of the RAF inhibitor, RG7204/PLX4032 (Plexxikon), found that approximately 75% of mutant B-RAF melanoma patients displayed responses by RECIST criteria (Flaherty *et al.*, 2009). Additionally, a phase III study of ipilimumab (Bristol–Myers Squibb), an anticytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) drug, improved overall median survival for metastatic melanoma patients that had received previous treatment (Hodi *et al.*, 2010). Although the outlook appears promising, major difficulties remain. For example, the majority of patients who initially responded to RG7204 have subsequently relapsed, raising concerns about acquired/secondary resistance. Also, despite the improved median survival data for ipilimumab, the response rate was only 11%. Hence, additional targeted therapies are needed urgently.

In this issue, Chen *et al*. focus on targeting c-*Myc*, a proto-oncogene that is well studied in cancer. c-*Myc* is a basic helix–loop–helix leucine zipper transcription factor that is highly expressed in melanoma (Ross and Wilson, 1998). Importantly for the current study, c-*Myc* depletion in melanoma cells induces cell cycle arrest and a senescence-like phenotype (Zhuang *et al.*, 2008). Conversely, overexpression of c-*Myc* in normal melanocytes inhibits mutant B-RAF-induced senescence (Zhuang *et al.*, 2008). These effects may result from alterations in the expression of several c-*Myc* transcriptional target genes that encode rate-limiting enzymes for dNTP metabolism (Mannava *et al.*, 2008).

Nanoparticle-based melanoma treatment strategies

Chen *et al*. (2010, this issue) used a c-*Myc* small interfering RNA (siRNA) depletion strategy combined with a unique liposomal nanoparticle formulation to increase siRNA stability and cell delivery. They developed nanoparticles formed from a DSAA carrier lipid, instead of the previously utilized DOTAP liposome particles, which were targeted specifically to melanoma cell sigma receptors via an anisamide ligand (DSAA AA+). Compared with DOTAP AA+ nanoparticles, the DSAA AA+ particles containing c-*Myc* siRNA increased cell delivery and c-*Myc* reduction *in vivo*. In addition, dose-dependent tumor growth inhibition was seen after intravenous administration of DSAA AA+ c-*Myc* siRNA particles in the syngeneic B16F10 mouse melanoma tumor model and in a human melanoma xenograft system. Perhaps the most striking finding was the complete growth inhibition of B16F10 mouse melanoma tumors when the DSAA AA+ c-*Myc* siRNA particles were administered in combination with paclitaxel chemotherapy. Interestingly, the biological properties of the DSAA carrier lipid itself seem to add an additional dimension to the potency of this melanoma treatment strategy. Empty DSAA nanoparticles were shown to increase reactive oxygen species (ROS) levels, decrease expression of the prosurvival protein Bcl-2, and increase apoptosis in B16F10 cells. Importantly, all treatments with DSAA nanoparticles were demonstrated to have low immunotoxicity. Taken together, these results highlight the potential of utilizing DSAA AA+ cationic nanoparticles to target gene expression as a new melanoma treatment option. These results, along with the possible chemotherapeutic drug combination avenues they open, may one day offer hope to patients with advanced melanomas, especially those who may have acquired resistance to other treatments.

Chen and colleagues (2010) are not the first to utilize nanoparticles as a siRNA delivery vehicle to target melanomas in preclinical assays. Tran *et al*. (2008) utilized topical applications of liposomal nanoparticles combined with ultrasound to promote B-RAF^{V600E}-selective and Akt3 siRNAs to penetrate into the skin microenvironment and inhibit the growth of mutant B-RAF melanoma cells in 3-D *in vitro* and xenograft assays. These studies utilized DOTAP/DOPE/DSPE-PEG(2000)-formulated nanoparticles that might lack the ROS-generating effects observed with the DSAA-formulated nanoparticles used in the Chen *et al*. experiments. In a separate study, investigators delivered systemically protease-activated receptor-1 siRNA incorporated into neutral DOPC liposomal nanoparticles (Villares *et al.*, 2008). Importantly, protease-activated receptor-1 siRNA-containing liposomes decreased growth and metastasis of human melanoma cells in nude mice.

Nonliposomal nanoparticles have also been used to deliver siRNAs and pharmacologic inhibitors into primary and metastatic melanomas. Similar to the Chen *et al*. study in this issue, Zamora-Avila and colleagues (2009) used the B16F10 mouse melanoma tumor model but instead focused on the treatment of melanoma lung metastases. They formulated Wilms' tumor gene 1 siRNA-polyethylenimine nanoparticles and introduced them into the murine lung via an aerosol-based delivery system. Strikingly, these nanoparticles decreased lung tumor burden and increased survival without lung tissue damage or acute inflammatory response (Zamora-Avila *et al.*, 2009). Yet another study expanded the scope of the use of nanoparticles to include pharmacological drug delivery with particular focus on the improvement of sustained and targeted drug release. Basu *et al*. (2009) derived nanoparticles composed of a hexadentate–polylactic acid–glycolic acid polymer conjugated to the MAP/ERK kinase pharmacologic inhibitor, PD98059. This formulation demonstrated increased inhibition of melanoma cell proliferation both *in vitro* and *in vivo* compared with traditional drug delivery methods (Basu *et al.*, 2009). In addition, PD98059-conjugated nanoparticles could synergize with cisplatin treatment to demonstrate enhanced antitumor activity in the B16F10 mouse melanoma model.

Concluding remarks

Given the scarcity of effective treatment options available for patients with advanced melanoma, the dawn of nanoparticle delivery systems could not come at a better time. Future studies in the field should focus on potential combinations of siRNA- and chemotherapeutic/ pharmacological inhibitor-containing nanoparticles because both formulations have been shown to increase delivery and effectivenesss. In addition, although syngeneic mouse models such as B16F10 are highly important in the development of cancer therapies, it would be advantageous to extend these studies into models more relevant to human melanoma progression. Specifically, the recent conditional B-RAF^{V600E}/PTEN-deficient metastatic melanoma mouse is an ideal disease model for preclinical studies of these nanoparticles (Dankort *et al.*, 2009). Additional studies with human melanoma xenograft mice, which allow for representation of a wide range of melanoma genetic backgrounds and staging, would also be of value.

In conclusion, increasing evidence from the past several years has revealed nanoparticles to have the potential to improve the efficacy of current treatments and/or to open doors to strategies such as siRNA targeting, which until now have been plagued with problems. The work of Chen *et al.* (2010) extends this area of research and demonstrates that targeting melanoma tumor cells with c-*Myc* siRNA packaged within the new DSAA nanoparticle, alone or in combination with chemotherapy, can inhibit melanoma tumor growth in several systems, offering hope for the development of potent and efficacious treatment strategies for advanced melanoma.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Basu S, Harfouche R, Soni S *et al.* (2009) Nanoparticle-mediated targeting of MAPK signaling predisposes tumor to chemotherapy. *Proc Natl Acad Sci USA* 106:7957–61

Chen Y, Bathula SR, Yang Q *et al.* (2010) Targeted nanoparticles deliver siRNA to melanoma. *J Invest Dermatol* 130:2790–8

Dankort D, Curley DP, Cartlidge RA *et al.* (2009) Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nat Genet* 41:544–52

Flaherty K, Puzanov I, Sosman J *et al.* (2009) Phase I study of PLX4032: proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. *J Clin Oncol* 27: 15S (Abstract 9000)

Hodi FS, O'Day SJ, McDermott DF *et al.* (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 363:711–23

Mannava S, GrachtchoukV, Wheeler LJ *et al.* (2008) Direct role of nucleotide metabolism in C-MYC-dependent proliferation of melanoma cells. *Cell Cycle* 7:2392–400

Ross DA, Wilson GD (1998) Expression of c-*Myc* oncoprotein represents a new prognostic marker in cutaneous melanoma. *Br J Surg* 85:46–51

Tran MA, Gowda R, Sharma A *et al.* (2008) Targeting V600EB-Raf and Akt3 using nanoliposomal-small interfering RNA inhibits cutaneous melanocytic lesion development. *Cancer Res* 68:7638–49

Villares GJ, Zigler M, Wang H *et al.* (2008) Targeting melanoma growth and metastasis with systemic delivery of liposome-incorporated protease-activated receptor-1 small interfering RNA. *Cancer Res* 68:9078–86

Zamora-Avila DE, Zapata-Benavides P, Franco-Molina MA *et al.* (2009) WT1 gene silencing by aerosol delivery of PEI–RNAi complexes inhibits B16-F10 lung metastases growth. *Cancer Gene Ther* 16:892–9

Zhuang D, Mannava S, Grachtchouk V *et al.* (2008) C-Myc overexpression is required for continuous suppression of oncogene-induced senescence in melanoma cells. *Oncogene* 27:6623–34

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Oxygenation State as a Driver of Myofibroblast Differentiation and Wound Contraction: Hypoxia Impairs Wound Closure

Chandan K. Sen[1] and Sashwati Roy[1]

Myofibroblasts are ubiquitous in the human body and may form from the differentiation of fibroblasts, epithelial cells, endothelial cells, and mononuclear cells, among others. Their clinical significance could be substantial, depending on biomedical context. Myofibroblasts help contract open skin wounds, but they could also be key drivers of fibrosis across numerous tissue systems and support tumor invasiveness. Understanding the molecular events underlying myofibroblast formation is significant for many human diseases. In this issue, Modarressi *et al.* address the significance of wound tissue hypoxia in impairing wound contraction by compromising myofibroblast formation. They present compelling evidence indicating tissue hypoxia conflicts with wound closure. We are reminded that correcting wound tissue hypoxia is critical for the tissue's response to other therapeutic interventions.

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In 1977, Packer reported that human diploid fibroblasts grown at 10% O_2 live longer than cells grown at the routine 20% O_2 (Packer and Fuehr, 1977). The field of cellular senescence was in its infancy, with the concept of the "Hayflick limit" reported in 1961. In 2003, Roy *et al.* (2003a) reported that growth arrest of fibroblasts caused by 20% O_2 was reversible, consistent with the current study (Modarressi *et al.*, 2010), and it was therefore concluded that exposure of cells to hyperoxic insult causes differentiation, but not senescence. Although it is standard practice to culture cells at an ambient O_2 concentration of 20% (i.e.,

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